CHARACTERIZATION OF SEDIMENT CONTAMINANTS ACUTELY LETHAL TO *DAPHNIA MAGNA*

By

SHERRY HAM RATZLAFF

Bachelor of Science

Radford University

Radford, Virginia

1984

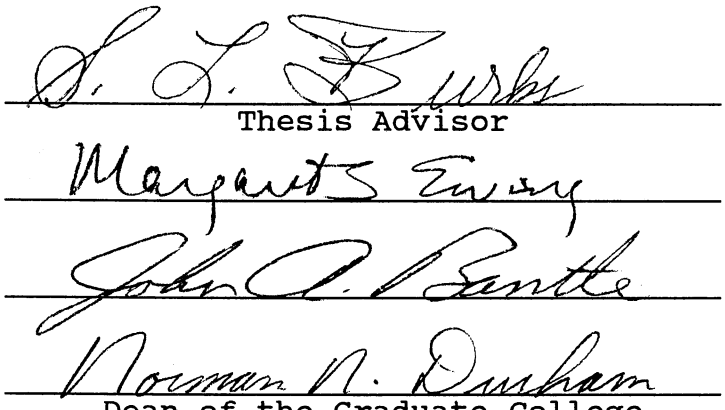
Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fullfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1988

CHARACTERIZATION OF SEDIMENT

CONTAMINANTS ACUTELY LETHAL

TO DAPHNIA MAGNA

Thesis Approved



Thesis Advisor

Dean of the Graduate College

ACKNOWLEDGEMENT

I would like to thank Dr. Bud Burks for guiding me to work on this interesting and meaningful project. His knowledge, advice, and kindness were greatly appreciated. I am also extremely grateful to Elaine Stebler for her help in this project and for her patience and skill in teaching me lab skills and culturing methods.

I am also thankful to Dr. Margaret Ewing and Dr. John Bantle for their advice and for reviewing my thesis, Sarah Kimball and Greg Smith for their invaluable help and advice, and to Greg Roberts and Sean Stebler for their time spent helping me in the field.

Gratitude is also extended to the Oil Refineries Waste Control Council. Though this project was not directly funded by them, some of the chemicals and much of the equipment used was previously purchased with these funds.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The primary objective of this project was to determine if sediments in a stream receiving oil refinery wastewater contained acutely lethal levels of contaminants. Historically, the wastewater in question has met or exceeded all National Pollution Discharge Elimination System permit criteria and has not exhibited acutely lethal effects to Daphnia magna in 48-hour static tests. If the sediments exhibited toxicity, a subsequent objective was to investigate methods to isolate and characterize major groups of acutely lethal contaminants.

In the past the major concern of environmental agencies in determining quality of water receiving industrial effluents was contaminant concentrations within the water column. It was suspected that considerable quantities of contaminants could be associated with or absorbed onto suspended particles and sediments. However, only recently have sediment and suspended particles become a major investigative focus for aquatic scientists. It has been found that sediments can store toxic chemicals at levels much higher than the concentration in the water column (Lowengart et al. 1987). Water may transport these

contaminated sediments well beyond the point of origin (Barth and Starks 1985). During transport, metals and organic compounds can slowly desorb from the sediment creating toxic effects in locations remote from the original source of contamination (Lee and Jones 1987).

Organic compounds with low molecular weights and compounds with aromatic or olefinic characteristics have higher solubilities in water (Wolfe 1987). As the lower molecular weight (more soluble), more volatile compounds disappear the heavier residual compounds increasingly partition with sediment or particles in the water. Thus, an accumulation of hydrophobic substances in the sediment may occur over many years.

Contaminant partitioning has been recognized to be a function of sediment characteristics, including grain size and organic content (Bahnick et al. 1980, Chapman 1986). For example, sorption of hydrophobic contaminants on sediment is closely related to the organic carbon content of the sediment (Karickhoff et al. 1979, Adams 1987). Lipophilic contaminants tend to associate with other lipophilic organic compounds or phase boundaries. Particle surfaces, the mud-water interface, and the air-water boundary may concentrate lipophilic compounds above ambient concentrations in water.

Lipophilic properties of chemical contaminants have already been implicated by a quantitative structure

activity relationship investigation as correlated with narcotic effects on aquatic organisms and with bioconcentration factors (Veith 1983, Lake et al. 1987). Logically, increasing lipophilicity would decrease equilibrium concentration in the water column and increase affinity of contaminants for suspended particles that ultimately are deposited in sediments.

Studies of polychlorinated biphenyl (PCB) content and release from sediments showed that highest concentrations of PCB's occurred in oily sediment and lowest concentrations in sandy sediment. However, the rate of PCB release from these sediments was inversely related to their concentrations; the sandy sediment released the greater quantity of PCB's. In addition, comparisons of bioassay results to direct sediment analysis indicated that only a small part of a wide variety of contaminants were available to test organisms (Jones et al. 1981).

Sediment bioassays can be useful in the identification of specific toxic compounds and facilitate development of methods for their removal from wastewaters before these waters are released into the environment. However, sediment toxicity is a relatively new concern and standardization of methods for sediment testing is still in the developmental stages. Many methods adopted for testing sediment toxicity were developed to assess the water quality impacts of hydraulically dredged sediments (Lee and Jones 1987). These methods involve eluting a sediment with

reconstituted water or sample site water and testing the elutriate.

An evaluation of elutriate toxicity testing found this method useful for predicting the release of contaminants from sediments that are stirred into the water column (Jones and Lee 1978). Although most water systems have appreciable mixing and dilution and static lab systems do not, high concentrations of the contaminant may be present because the sediment is mixed with a limited volume of water. Thus, the elutriate method tests for potential worst case conditions and its values are not exceeded in nature (Lee and Jones 1987).

Water contamination from a petroleum refinery outfall may originate from a variety of sources and introduce a complicated array of toxic compounds. Petroleum refineries use water to remove inorganic salts by washing crude oil stock (Burks 1982). This introduces dissolved or emulsified organic compounds into the water. Water is also used to strip undesirable constituents such as hydrogen sulfide and ammonia from the crude oil fractionation units. Condensed water from stripper units is contaminated with volatile organic chemicals produced by cracking of heavier hydrocarbons. Phenolics may also be present in high concentrations in stripper condensate. Chemicals used within the refinery for controlling scale and corrosion in cooling towers or reduction of frothing in desalters may

also contribute to contamination of the wastewater. Thus, oil refinery wastewaters may contain a complex mixture of hundreds of compounds with different chemical properties and deleterious toxic effects on aquatic organisms.

The complexity of oil refinery wastewater generally precludes toxicity testing of each individual compound in the effluent. However, tests have been conducted by chemically fractioning the effluent into less complex components (Guerin et al. 1978, Parkhurst et al. 1979, Reece and Burks 1984, Chian et al. 1985, Lopez-Avila et al. 1986, Anderson-Carnahan and Mount 1987). Fractionation followed by bioassay of each fraction has been found useful in identification of toxic constituents of petroleum wastewater. Toxicity of each fraction relative to the toxicity of the unfractioned sample indicates the effect of the fractionation and provides information on the nature of the toxic constituents.

Chelation, filtration, activated carbon adsorption, and distillation have been used as fractionation techniques to remove toxic contaminants in wastewater analysis (Reece 1984). Chelation is useful to determine if toxicity is caused by cationic toxicants such as heavy metals. A strong chelating agent such as ethylenediaminetetraacetate (EDTA) produces relatively nontoxic complexes with many metals and makes them biologically unavailable (Anderson-Carnahan and Mount, 1987). Filtration provides information on toxicants associated with dissolved versus suspended

(filterable) material. Activated carbon has been shown to selectively remove non-polar organics from water by adsorption. After treatment with activated carbon, the remaining contribution by polar organic compounds, ammonia, and hydrogen sulfide to the toxicity of the wastewater can be evaluated. Distillation removes low molecular weight, volatile compounds and allows an evaluation of their toxic effects. The residual non-volatile fraction may also be evaluated for toxicity.

Biological uptake rate by passive diffusion of some trace organic contaminants in water is dependent on the presence of ionizable moieties such as carboxyl and/or amino groups (Morrison and Boyd 1983). Adjustment of pH can change the relative polarity and thus the relative deleterious effects of these contaminants. Acidification of wastewater to pH 4 prior to carbon adsorption allows an evaluation of the contribution of phenolics and other weak organic acids to toxic effects. Conversely, toxicity tests of wastewater adjusted to an alkaline pH prior to activated carbon treatment may indicate effects of ionizable basic compounds such as organic amines. Different levels of toxicity may also occur among acidic, basic, and neutral portions of distilled fractions. For example, at an acidic pH, low molecular weight ionizable weak organic acids and phenolics will volatilize more readily. Both the volatile and non-volatile fractions may then be analyzed or tested for toxic effects.

Fractionation procedures may change the physical or chemical nature of the test material and alter the toxicity. Therefore, fraction preparation should result in minimal or at least interpretable impact on the test material (Epler 1978). If the toxicity is assumed to be additive, results are interpretable. If the sum of the fraction median lethal times (LT50s) equals the LT50 of the unfractioned sample then the fractionation procedures probably do not alter the toxicitiy of the fractions in question (Parkhurst et al. 1979).

Ammonia, sulfides, cyanides, and phenolics alone or in combination are suspected to be major contributors to toxicity in petroleum refinery effluent and process streams (Mathews and Myers 1976). Treatment of highly volatile fractions of petroleum refinery effluent with activated carbon resulted in a sharp decrease in acutely lethal effects on daphnids in a study by Reece (1983). In this study, survival increased when the distillate was treated with exchange resin which removed cations. Distillate with high ammonia levels was tested at acidic, basic, and neutral pH. The percent unionized ammonia, the more toxic form, was found to increase tenfold with every pH unit increase. Other toxic components were characterized as base-neutral polycyclic aromatic hydrocarbons with a molecular weight between 180 and 300. In general, volatile organic compounds have been found to be the toxic agents in petroleum wastewater (Dorris et al. 1972, Reece 1983).

Complex mixtures like oil refinery wastewaters are generally poorly defined and are composed of continuously changing mixtures of compounds. However, researchers are gaining some insight into this wastewater composition. Similar analyses of bioavailable toxicants from sediment are needed. Fractionation appears to be an effective method to simplify these mixtures to a degree such that toxic components can be isolated. Once the most toxic fractions are isolated and characterized, their environmental significance and potential removal can be investigated.

CHAPTER II

METHODS

Sediment was collected from Omaha Creek, Kay County, Oklahoma, south of a county road bridge, approximately 30 m south of an oil refinery wastewater outfall (NE 0.25 of NE 0.25 of Sec 9, T25N, R2E). Control sediment was collected from Stebler Creek, Payne county, Oklahoma, (N 0.5 of S.W. 0.25 of Sec 27, T17N, R3E). Sediment was collected and fractioned during August and again in October, 1987.

The sediment sample was collected with a 10.16-cm (4-inch) corer from water less than 60 cm deep and transported in sealed plastic containers on ice. Samples were stored in the dark at 4 C with no more than 5 cm of water overlying the surface of the sediment. All fractionations and tests were performed within a recommended two week period to prevent potential sample degradation over time (US EPA/US Army Corps of Eng. 1977). Particle size analysis and total organic carbon content was determined for all sediment samples (Day 1965, Gaudette et al. 1974).

Glassware was rinsed in acetone and nitric acid before being washed in detergent and hot water. It was then

rinsed in hot tap water three times and double rinsed with distilled water.

The sediment was eluted with reconstituted water according to a modification of the Nebeker et al. (1984) liquid phase elutriate test procedure. Reconstituted water used in this study was prepared according to EPA specifications (Peltier and Weber 1985). Sediment was mixed in a volumetric sediment-to-water ratio of 1:2 at room temperature in a large glass jar; preliminary tests indicated acute toxicity at this ratio. The sediment was measured by volumetric displacement. One hundred ml reconstituted water were placed in a graduated beaker and sediment added to obtain a total volume of 400 ml. The beaker was then filled to the 900 ml mark with reconstituted water and mixed using a glass rod. This produced 900 ml of slurry with a final ratio of one volume sediment to two volumes water, which provided about 600 ml sediment elutriate.

The slurry was stirred slowly by hand for 15 minutes. Approximately equal amounts of the stirred slurry was added to each of six 250 ml polycarbonate centrifuge bottles and centrifuged at 10,400 X g (average) for 15 minutes at 20 C using a Sorvall Superspeed RC2-B centrifuge with a model GSA rotor head. Preliminary testing indicated this speed and time combination effective for removal of suspended particles. Each batch of elutriate was pooled. It was maintained at 4 C until used within two weeks. A

portion of reconstituted water was also centrifuged under the same conditions and control sediment was prepared using an identical procedure. Alkalinity was measured by the titrimetric method using Hach ^{R} chemicals (U.S. EPA 1979). Hardness as total mg/l of $CaCO_3$ was measured by the titrimetric, EDTA method (U.S. EPA 1979). The elutriate was then ready for use in fractionations and the baseline toxicity test.

Static Acute Daphnia magna Toxicity Tests

Baseline elutriate (unfractioned) was tested using a modification of the liquid phase elutriate test presented by Nebeker et al. (1984). The baseline elutriate provided a comparison for evaluating relative effects of the fraction tests. Test organisms, Daphnia magna, were cultured according to standard methods (APHA 1980).

Procedure for the Baseline Elutriate Test

The baseline elutriate test included 3 controls. Four components were tested:

1) baseline elutriate
2) control sediment elutriate
3) centrifuged reconstituted water
4) reconstituted water

Two replicates of each component were used. For each replicate, 100 ml of solution to be tested was added to a 150 ml beaker. Preliminary tests yielded dubious results

because daphnids floated in test solutions. Oils in the sediment and elutriate decrease the effective density of daphnids and cause them to float (Buikema et al. 1980). A screen device made of 100-micron mesh Nitex and a stainless steel ring was used in each beaker to eliminate floaters when this was a problem (Dean and DeGraeve 1986). Beakers were aerated for 0.5 hour before pH and dissolved oxygen were measured and recorded. Daphnids from three to five days old were then added to each beaker one at a time until each beaker contained ten daphnids. Three-to five-day-old daphnids were used as test organisms because they are larger and easier to see in the turbid water than are younger individuals (Nebeker et al. 1984).

Acute toxicity was evaluated by using timed lethality tests. In these tests the relative toxicity of the effluent was measured in terms of the time required to elicit a given response from exposed daphnids. The percentage mortality, on a probit scale, was graphed against the exposure time, on a logarithmic scale. A straight line was fitted to the graph points and the median lethal time (LT50) was interpolated from the point where the regression line crossed the 50% mortality intercept. In this way relative toxicities may be evaluated (Anderson-Carnahan and Mount 1987).

Daphnid mortality was determined every 30 minutes during the first and second hour. Thereafter, mortality was determined at 2, 4, 8, and 24 hours. At each

observation, the number of dead daphnids were recorded. The criteria for death were inability to swim and remaining on the bottom of the beaker with minimal or no movement. The test was considered invalid if more than 10% of the control daphnids died.

Fractionation

Activated carbon adsorption at pH 4, 7, and 12, distillation at pH 4, 7, and 12, 0.45 uM filtration, heating at pH 4, chelation, and degradation were used to produce separate fractions of sediment elutriate (Figure 1.). Each fraction was tested using baseline elutriate toxicity testing procedures.

Activated Carbon Adsorption

Three 200-ml samples were obtained from the baseline elutriate. The pH of the first sample was adjusted to pH 4 by dropwise addition of 1.0 N hydrochloric acid, the second sample was adjusted to a pH of 12 with 1.0 N sodium hydroxide, and the third sample was adjusted when necessary to pH 7. One gram of granular activated carbon was added to each sample and stirred with a Teflon coated magnetic stir bar for 15 minutes. Elutriate was then filtered through a 0.45-uM glass fiber filter to separate the elutriate from activated carbon. Following carbon adsorption, samples were restored to the original pH and tested for toxicity.

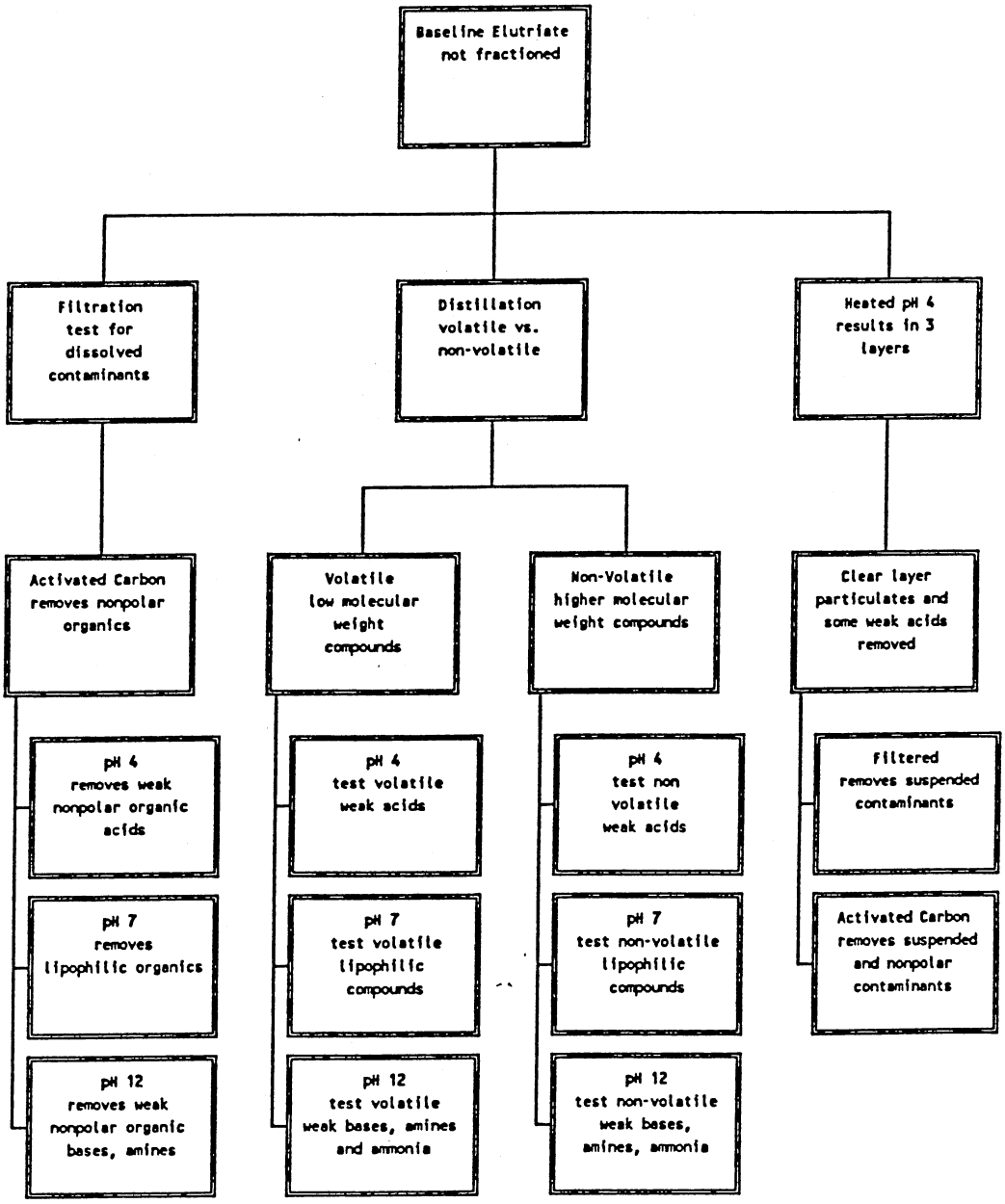


Figure 1. Flowchart for fractionation scheme. (chelation and degradation excluded)

Distillation

One liter of baseline elutriate was adjusted to pH 4, one liter to pH 12, and one liter to pH 7 as described in the activated carbon adsorption procedure. Each liter was distilled according to a simple distillation procedure. Twenty percent of the sample was distilled to avoid dilution of the distillate. Control elutriate was prepared in the same manner. Each distillate (volatile portion) was returned to the original pH and tested according to baseline test procedures. The portion not distilled (non-volatile) from each respective distillate was also adjusted to the original pH and tested.

0.45 uM Filtration

Two hundred ml of baseline elutriate was passed through a 47-mm diameter Gelman type A/E glass fiber filter (without organic binder) which should retain particles greater than 0.45-uM. Dissolved compounds were then tested for toxicity.

Heating at pH 4

About one liter of the elutriate was heated to 40 C at pH 4. At this temperature a precipitate formed leaving 3 layers; a thin oily top layer, a clear middle layer (about 75% of total volume), and a dark, opaque precipitate on the bottom (about 24% of total volume). The clear layer was

carefully removed. This layer was then tested, filtered and tested, and treated with activated carbon, filtered, and tested. An attempt was made to remove the oily layer. The oily layer was tested in August only.

EDTA Chelation

A 0.1 Molar (M) solution of EDTA was prepared by dissolving 37.23 g disodiumethylenediaminetetra-acetatedihydrate in 1 liter distilled water in a polyethelene container (APHA 1980). Successive small measured amounts of EDTA were added to aliquots of elutriate as recommended by Anderson-Carnahan and Mount (1987). The concentration at which EDTA becomes toxic is a function of the sample. EDTA is less toxic in samples with relatively high hardness. For this reason, ten, 5-ml aliquots were used. To the first, 0.05 ml (1 drop) of EDTA was added, to the second sample 0.15 ml (3 drops) were added, to the third, 0.25 ml (5 drops), and so on until the tenth elutriate sample received 0.95 ml (19 drops). Daphnid floaters in this test were a problem because of the small size of the test vials, necessary due to the number of dilutions required. Screens could not be used. The results from this test were somewhat questionable because of floaters.

Degradation

In this test, stability of sediment toxicants was tested. Results from this test should indicate whether toxicants in the sediment were biodegradable, photolyzable, and/or oxidizable (Anderson-Carnahan and Mount 1987). Four hundred ml of baseline elutriate were set aside in a covered glass container for degradation. Two hundred ml was stored in light at room temperature and 200 ml stored in dark at room temperature. Similar portions of control elutriate were stored in light and dark. Degraded elutriate was tested for toxicity after 2, 4, 8, and 24 days. Dissolved oxygen was not monitored during degradation and the samples could have become anoxic. For this reason the results of this fraction were not considered reliable.

Organic Compound Analysis

A 100-ml aliquot of each fraction was extracted in a 250-ml separatory funnel with three, 60-ml portions of methylene chloride. They were dried over sodium sulfate, concentrated, and analyzed by gas chromatography. Only the fractions collected in October were analyzed. About one ul of the concentrate was injected into a Tracor ^{R} 500 gas chromatograph (GC) system with a 3% ov-1 on Supelcoport ^{R} 80/100 standard (6.35 mm x 1.83 m) packed glass column. The system was programmed to an initial temperature of 150

C with 6 C increase each minute up to 250 C and a 10 minute final hold. A flame ionization detector was used to quantify compounds detected. The on-column concentration was determined by comparing the area response of the unknown with different area responses of a respective decane (C_{10} to C_{19}) standard using a regression equation. Each fraction was spiked with 200 ng deuterated anthracene (d_{10}) per ul sample to allow accurate comparison of each fraction. All retention times were determined relative to D_{10}. Detection limits were based partially on their detectibility by GC. Once the on-column detection limit was determined, then the volume injected on column, total volume of sample in tube, and volume of water extracted were entered into the equation (Smith 1987):

Detection Limit ug/100 ml elutriate =

$$\left[\frac{\text{ug respective component of } D_{10} \text{ det. on col.}}{\text{ul sample injected on column}} \times \frac{\text{ul sample}}{\text{in tube}}\right]$$

Statistical Analysis

The EPA Toxdat multimethod computer program was used to calculate the LT50 and 95% confidence intervals using probit methods. Heirarchical clustering was performed using Biomedical Programs (BMDP2M). Clusters were based on similarity of peak retention times in each fraction (Morgan et al. 1987). Principle component analysis was used to simplify the data set to a smaller number of uncorrelated

components (Wolff and Parsons 1983). This information was then used to examine the relation of toxicity correlation to peak distribution.

CHAPTER III

RESULTS

All controls with the exception of the degradation component had Daphnia magna LT50's greater than 48 hours. GC analysis detected peaks in the controls only when the extract was concentrated to less than one ml.

The baseline extracts from August and October were similar with LT50's at 4.0 and 3.1 hours, respectively (Figures 2. and 3.). The toxicity of the filtered fraction was not as consistent from August to October. The August fraction resulted in an LT50 of 29.8 hours, versus an LT50 of 9.7 hours for the October fraction. When the baseline was heated at pH 4 to 40 C, the toxicity was slightly increased in August with an LT50 of 2.8 hours. In October this procedure slightly reduced toxicity to 11.8 hours.

Heating the baseline at pH 4 resulted in 3 layers: a thin oily layer at the top, a relatively clear middle layer, and an opaque, flocculent bottom layer. The sample was further fractioned by siphoning off the clear middle layer which was then filtered. This greatly reduced toxicity with LT50's at 31.5 hours and 48+ hours in August and October, respectively. In addition to filtration, the

clear layer was treated with activated carbon. This fraction was very similar to the previous fraction with LT50's of 31.5 hours for August and 48+ hours for October, again indicating greatly reduced toxicity.

In August only, an attempt was made to siphon the extremely thin oily layer from the pH 4 heated baseline. This fraction resulted in a low LT50 of 1.6 hours.

When the entire baseline elutriate was treated with activated carbon and filtered at pH 4, LT50's were 30.6 hours (August) and 13.9 hours (October). At pH 7 toxicity increased with LT50's of 13.9 hours (August) and 6.8 hours (October). At pH 12 LT50's were 12.3 and 5.7 hours, in August and October samples, respectively.

The volatile fractions, at all pH's, produced the lowest LT50's ranging from 1.2 to 2.3 hours in August and in October. The nonvolatile fractions also produced low LT50's for all pH's ranging from 3.1 to 6.1 hours in both August and October samples.

Results from chelation indicated that metals were not contributing to toxicity. Increasingly higher but erratic, mortality was indicated at higher EDTA concentrations. No reduction in toxicity at any concentration was observed. However, the small quantities necessary and resulting small vials used for testing made it impossible to control floaters. Therefore, the results of this test were not considered reliable (Appendix C.).

The degradation tests indicated that the baseline toxicity decreased over time. However, there was a great deal of mortality in one of the controls, probably due to a contaminated container, also, oxygen levels were not observed. For these reasons the degradation test results were not considered reliable (Appendix D.).

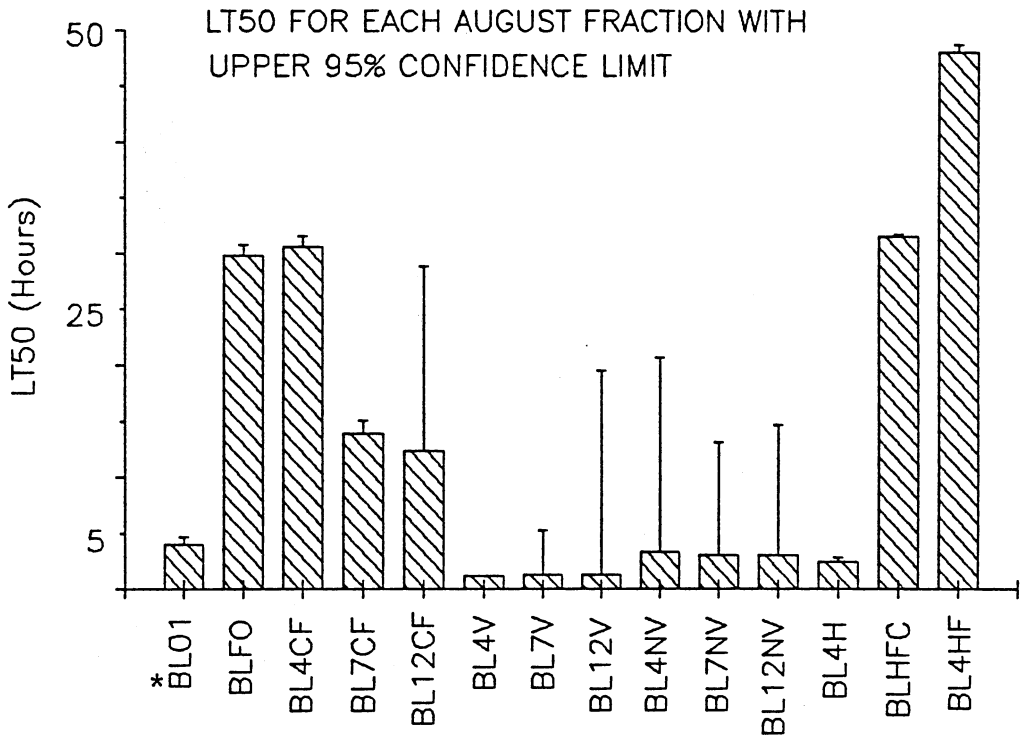


Figure 2. Bargraph for August fraction LT50's.

*Refer to page 42 for explanation of abbreviations.

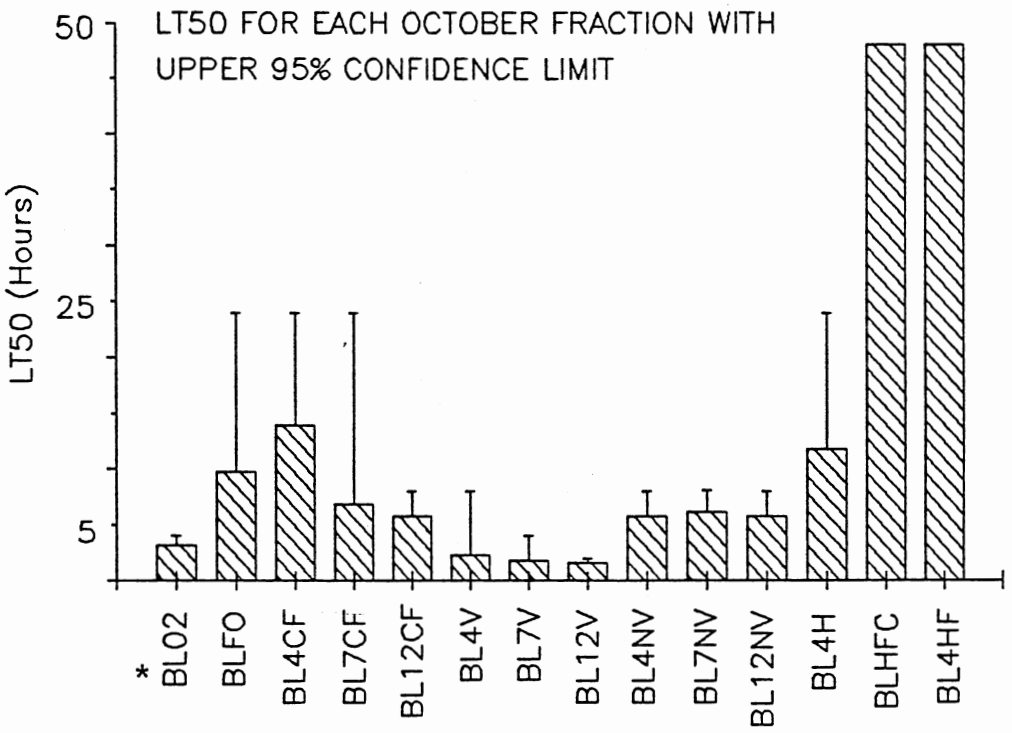


Figure 3. Bargraph for October fraction LT50's.

*Refer to page 42 for explanation of abbreviations.

CHAPTER IV

DISCUSSION

In August no benthic invertebrates were observed inhabiting the sediments during collection. The sediment consistency was very stiff and clumped. A particle analysis indicated 12% sand, 65% silt, and 23% clay. Organic carbon content was not measured for August.

Toxicity was greatly reduced in August by filtration only (Table I.), which indicates the toxic constituents were probably either bound to suspended particulates or in an emulsion. However, even with a large reduction in toxicity, this fraction was still acutely toxic. It was likely that some of the contaminants associated with the sediment desorbed and became dissolved in the water.

The toxicity was not much different when the baseline elutriate was reduced to pH 4, treated with activated carbon and filtered as compared with the filtered-only fraction (Figure 2.). Thus, dissolved weak organic acids were apparently not the major contributers to toxic effects. At pH 7 and 12, activated carbon treatment and filtration only slightly reduced toxicity, reinforcing the

CHAPTER IV

Discussion

In August no benthic invertebrates were observed inhabiting the sediments during collection. The sediment consistency was very stiff and clumped. A particle analysis indicated 12% sand, 65% silt, and 23% clay. Organic carbon content was not measured for August.

Toxicity was greatly reduced in August by filtration only (Table I.), which indicates the toxic constituents were probably either bound to suspended particulates or in an emulsion. However, even with a large reduction in toxicity, this fraction was still acutely toxic. It was likely that some of the contaminants associated with the sediment desorbed and became dissolved in the water.

The toxicity was not much different when the baseline elutriate was reduced to pH 4, treated with activated carbon and filtered as compared with the filtered-only fraction (Figure 2.). Thus, dissolved weak organic acids were apparently not the major contributers to toxic effects. At pH 7 and 12, activated carbon treatment and filtration only slightly reduced toxicity, reinforcing the

possibility that particulate bound weak organic acids were the toxic components.

The volatile fractions were the most toxic. LT50's were about the same for pH 4, 7, and 12. All were slightly more toxic than the unfractioned baseline elutriate. The non-volatile fractions were also very similar in toxicity at pH 4, 7, and 12 and slightly more toxic than the unfractioned baseline elutriate, though less toxic than the volatiles. LT50's from non-volatile and volatile fractions in August appeared to be additive, i.e., their sum was approximately equal to the unfractioned baseline elutriate LT50. Thus, it may be assumed that the distillation procedure did not change the chemical characteristics of the August baseline elutriate. With this and the absence of toxicity reduction, it may also be assumed that the toxic substances could not be isolated by thermal characteristics, i.e. they occurred in both volatile and non-volatile fractions.

Heating at pH 4 produced 3 layers in the baseline elutriate, a top oily layer, a relatively clear middle layer, and a bottom flocculent layer. The middle layer was difficult to siphon off. Even slight agitation forced the bottom layer to go back into solution. Some mixing occurred during this first attempt at siphoning and was most likely the cause of the resulting toxicity. The increase in toxicity was perhaps due to variation between tests.

The clear layer was siphoned off and filtered. This produced about the same reduction in toxicity as the filtered only and activated carbon treated-filtered fraction indicating again that particulate bound weak acids may be toxic components in the baseline sediment elutriate.

With activated carbon treatment of the clear filtered layer, acute toxicity was apparently removed. Although the difference between the toxicity of the clear layer filtered and the clear layer treated with activated carbon and filtered fractions may also be due to variation between tests, it may indicate that toxicity is due to some dissolved nonpolar organic compounds in addition to particulate bound weak organic acids. This may also be reinforced by the slight decrease in toxicity between the pH 4-carbon treated-filtered fraction and the filtered only fraction.

During the October sediment collection several chironomids were observed. The sediment had the same consistency as was found in August though of a slightly lighter color. Particle size analysis indicated 81% sand, 13% silt, and 6% clay. These results were questionable due to spillage. The control sediment consisted of 96% sand, 2% silt, and 2% clay. There was 7% organic carbon in the refinery sediment and 1.2% in control sediment.

The October baseline elutriate toxicity was not much reduced by filtration only (Figure 3.). The suspended

particulates were possibly of a much smaller size and filtration did not clear the elutriate as it did with the August sample. The filtered fraction was opaque in October. Suspended particulates are again indicated to be related to toxicity.

Treatment of October elutriate with activated carbon and filtration at pH 4 resulted in more of an increase in the LT50 than filtration alone caused. As in the respective August fractions this may indicate weak organic acids bound to particulates as a major toxic component with some contribution also due to dissolved nonpolar organic compounds. Also, as in August, the same treatment at pH 7 and 12 resulted in very similar and more toxic fractions than at pH 4. Weak bases were probably not a major component of contamination.

In October as in August the volatile fractions from distillation were the most toxic. Fractions tested at all pH's were very similar with those at pH 4 slightly less toxic than the others. Of course, this too may be a result of testing variability and not a true indication of detoxification because of the very small differences among all pH's. The non-volatile fractions were also very similar at varying pH's and indicate little to no effect compared with toxicity of the baseline elutriate. These LT50's were not additive as in the August distillation which might indicate that the procedure broke down larger hydrocarbons in the elutriate into smaller, more volatile

compounds. However, as in the August distillation, it appears that little or no toxicity reduction was due to volatilization of sediment elutriate.

Heating at pH 4 in October produced identical layering to that in the August sample. However, this was the second attempt at siphoning; the clear layer was removed more precisely, which may be reason for the slight decrease in toxicity. Again, the clear layer, cleared by coagulation of particulates and the consequent decrease in toxicity point to particulates or emulsions as causative agents.

Filtration of the clear layer removed acute toxicity as did carbon treatment and filtration of the clear layer. Filtration, as before, appeared to be the most effective technique for toxicity reduction which would indicate that particulates and weak organic acids were the apparent sources of toxicity.

Some contaminants of this sediment elutriate were probably capable of desorbing from particulates and becoming dissolved in the water column in a toxic form. Particulate size may change in response to high water flow and scouring activities and filtration will not always remove toxicity. The presence of oil is conducive to formation of an emulsion. Also, the August sediment may have formed more of an emulsion, which would explain why filtration was more effective. In both August and October, the filters were very oily and blackened after filtration of only 200 ml, which required several hours, another

indication that an oily emulsion was filtered out taking some toxic compounds with it.

Activated carbon and filtration appeared to have a consistent effect on elutriate toxicity over time, although the particular character of the sediment and resulting toxic effects changed. Each October fraction subjected to this treatment was about twice as toxic as in August (Table I.). Filtration apparently removed much more toxicity than the activated carbon, although the carbon very likely contributed slightly to detoxification. The molecular size, polarity, and concentration of adsorbate in solution affects the carbon adsorption process (Smith 1987). The size of the organic compound and its ability to enter the internal pore is the basis of this process. Larger molecules are restricted to external carbon pores and could block micropores. In this way adsorption efficiency may be reduced. Since refinery sediment and elutriate have an oily quality, they probably contain many large molecules and compounds, which may reduce effectiveness of activated carbon to reduce toxicity as was expected.

Distillation test results also remained fairly consistent. Although distillation did decrease toxicity very slightly in October, it did not increase LT50's in August. Distillation is apparently not a major effective treatment for sediment detoxification.

TABLE I

LT50 TABLE FOR SELECTED FRACTIONS
(IN HOURS)

	*August	October
BL01	4.0	3.1
BLFO	29.8	9.7
BL4CF	30.6	13.9
BL7CF	13.9	6.8
BL12CF	12.3	5.7
BL4V	1.2	2.3
BL7V	1.3	1.8
BL12V	1.3	1.6
BL4NV	3.3	5.7
BL7NV	3.0	6.1
BL12NV	3.0	5.7
BL4H	2.8	11.8
BL4HF	31.5	48+
BL4HFC	48+	48+
BLOIL	1.6	-

*Refer to page 42 for explanation of abbreviations.

Layer formation resulting from heating at pH 4 is probably due to the emulsion breaking which releases particulates from suspension. For this reason it is suspected that the emulsion with its associated oily droplets and particulates with contaminants bound to them could be a major cause of lethality.

Though every effort was made to prevent forming an emulsion during preliminary development of elutriate preparation, it always appeared to form. Centrifugation at high RPM's and for long periods did not change opacity and oiliness of elutriate. Extreme care and gentle mixing of the sediment with reconstituted water made no difference in emulsion formation. It seems this sediment has a propensity to form an emulsion and would probably do so under natural sediment disturbances. Given the observation that some daphnid mortality was possibly due to the physical damage incurred by the oily particulates, inhibiting appendage movement and maybe clogging respiratory openings, emulsion formation is considered to contribute to toxicity here. Physical effects of toxic waters have recently been cited as the cause for toxicity in fish (Chapman 1987).

In August, only the baseline elutriate and the oily layer were analyzed by GC. A GC analysis was conducted on all October fractions. The August and October baseline elutriate chromatograms were very similar (Figure 4.).

These chromatograms are probably even more similar than the cluster analysis indicates (Figure 5.). Various peaks are indicated on the chromatogram of the October baseline samples but the GC integrater was unable to record retention times for them (Appendix A). Thus, these peaks (which correspond to August peaks) were not included in the cluster analysis program.

GC analysis resulted in peaks A to Z (Appendix A and B). Peaks A, B, E, O, N, P, D, and R appear to be major peaks. There were large amounts of peak A, with a relative retention time (RRT) of 0.420, in the volatile fractions. This peak was also found in August and October baseline samples and in moderate amounts in the baseline elutriate heated at pH 4, the resulting similarities of these fractions are indicated in Figure 5. Fractions containing peak A had toxic levels from 1.6 to 11.8 hours (Table II.)

TABLE II

FRACTIONS CONTAINING PEAK A

Peak A Found in:		
*fraction	LT50	amount
BLO1	4.0	med.
BLO2	3.1	med.
BL4H	11.8	med.
BL4V	2.3	high
BL7V	1.8	high
BL12V	1.6	high

*Refer to page 42 for an explanation of abbreviations.

Peak B (RRT = 0.563) was also found in very high amounts in the volatile fraction and in moderate quantities in the August and October baselines, and in baseline heated at pH 4 (Table III.).

TABLE III

FRACTIONS CONTAINING PEAK B

Peak B Found in:		
*fraction	LT50	amount
BLO1	4.0	med.
BLO2	3.1	med.
BL4H	11.8	med.
BL12NV	5.7	low
BL4V	2.3	high
BL7V	1.8	high
BL12V	1.6	high
BLHCF	48+	trace
RECON	48+	trace
BLOIL	1.6	low

*Refer to page 42 for explanation of abbreviations.

Trace or very low amounts of this peak were found in non-volatile-pH 12 baseline, heated-filtered-carbon treated-pH 4 baseline, oily layer of the baseline sample, and in reconstituted water. Chromatograms of non-volatile-pH 4 and non-volatile-pH 7 baseline samples have small flat peaks in this area but not enough quantity to be picked up by the integrator (Appendix A). All fractions, with the exception of heated-filtered-carbon treated-pH 4 baseline samples and reconstituted water, with peak B were toxic with LT50's from 1.6 to 11.8 hours. Heated-filtered-

carbon-pH 4 treated baseline and reconstituted water both had LT50's at 48+ hours and contained only trace amounts of peak B. Again, cluster analysis demonstrates similarities of these fractions in all but the two fractions containing trace amounts of Peak B, which were also much less toxic (Figure 5.).

Peak E (RRT = 1.125) was found in the August and October baseline samples, heated-pH 4 baseline samples, and the baseline oily layer sample. Reconstituted water, the baseline oily layer fraction, and all non-volatile fractions contained small amounts of peak E. Large quantities were found in all volatile fractions (Table IV.).

TABLE IV

FRACTIONS CONTAINING PEAK E

Peak E Found in:		
*fraction	LT50	amount
BL01	4.0	med.
BL02	3.1	med.
BL4H	11.8	med.
BL4NV	5.7	low
BL7NV	6.1	low
BL12NV	5.7	low
BL4V	2.3	high
BL7V	1.8	high
BL12V	1.6	high
RECON	48+	low
BLOIL	1.6	low

*Refer to page 42 for explanation of abbreviations.

All fractions containing peak E in at least medium amounts were clustered together as similar with respect to relative retention times. They also have low LT50's. Peaks N, O, and P were found in moderate amounts only in the volatile fractions with the exception of peak N which was also found in the volatile Stebler Creek-pH 12 in trace amounts.

TABLE V

FRACTIONS CONTAINING PEAKS N, O, AND P

Peak O Found in:			Peak N Found in:		
*fraction	LT50	amount	*fraction	LT50	amount
BL4V	2.3	med.	BL4V	2.3	med.
BL7V	1.8	med.	BL7V	1.8	med.
BL12V	1.6	med	BL12V	1.6	med.

Peak P Found in:		
*fraction	LT50	amount
BL4V	2.3	med.
BL7V	1.8	med.
BL12V	1.6	med.

*Refer to page 42 for explanation of abbreviations.

Peaks N, O, P, and A, major peaks of the volatile fractions, all have expected short retention times. The chromatograms of all volatile fractions were very similar (Appendix A.). Similarity of these fractions are also indicated by cluster analysis (Figure 5). No peaks were indicated afer E in the volatile fractions indicating that that these peaks did not volatilize or possibly were cracked and formed new compounds or contributed to

compounds already present, such as peaks B and C (Appendix A).

Peak D was found in the August and October baseline samples, heated-pH 4 baseline samples, and all volatile fractions (Table VI). All of these fractions were toxic with LT50's from 1.6 to 11.8 hours.

TABLE VI

FRACTIONS CONTAINING PEAK D

Peak D Found in: *fraction	LT50	amount
BL01	4.0	med.
BL02	3.1	med.
BL4H	11.8	med.
BL4V	2.3	high
BL7V	1.8	high
BL12V	1.6	high

*Refer to page 42 for explanation of abbreviations.

Peak R (RRT = 0.747) was found in large quantities in all fractions that were filtered (Table VII). Thus, R could be a result of filtration. Perhaps these fractions do not appear to have as much in common in the cluster analysis as fractions associated with previously mentioned peaks for this reason.

TABLE VII

FRACTIONS CONTAINING PEAK R

Peak R Found in: *fraction	LT50	amount
BL4H	11.8	med.
BL4V	2.3	med.
BL7V	1.8	high
BL12V	1.6	high
BL4CF	13.9	low
BL7CF	6.8	med.
BL12CF	5.7	low
BLFO	9.7	low
BL4HF	48+	low
BL4HCF	48+	low

*Refer to page 42 for explantion of abbreviations.

Although all of these peaks apparently have some relationship to toxicity of the baseline elutriate, the major toxic effect probably was contributed by a complex mixture of heavier hydrocarbons. This was indicated by the many varied, small and indistinct peaks at the higher retention times and the high correlation of total detected organic compounds.

A complex mixture of large hydrocarbons, possibly weakly acidic in nature and the oiliness associated with an emulsion appear to contribute to the acute lethality of this sediment elutriate. The effluent from the refinery, although not considered acutely toxic, apparently contains at least small amounts of compounds which accumulated in the sediment over time. These sediment-bound toxic compounds appear to be closely associated with particulates

as may be expected. However, it may be that these toxic compounds can desorb from the sediment and possibly contribute to toxicity of the water column.

Further studies regarding pH adjustment and filtration along with GC/Mass Spectrometry analysis are recommended in order to more accurately describe constituents responsible for toxicity. Once identified, or more closely characterized, attempts may be made to remove them from the effluent, eliminating toxic accumulation in sediment and its potential threat to the aquatic environment.

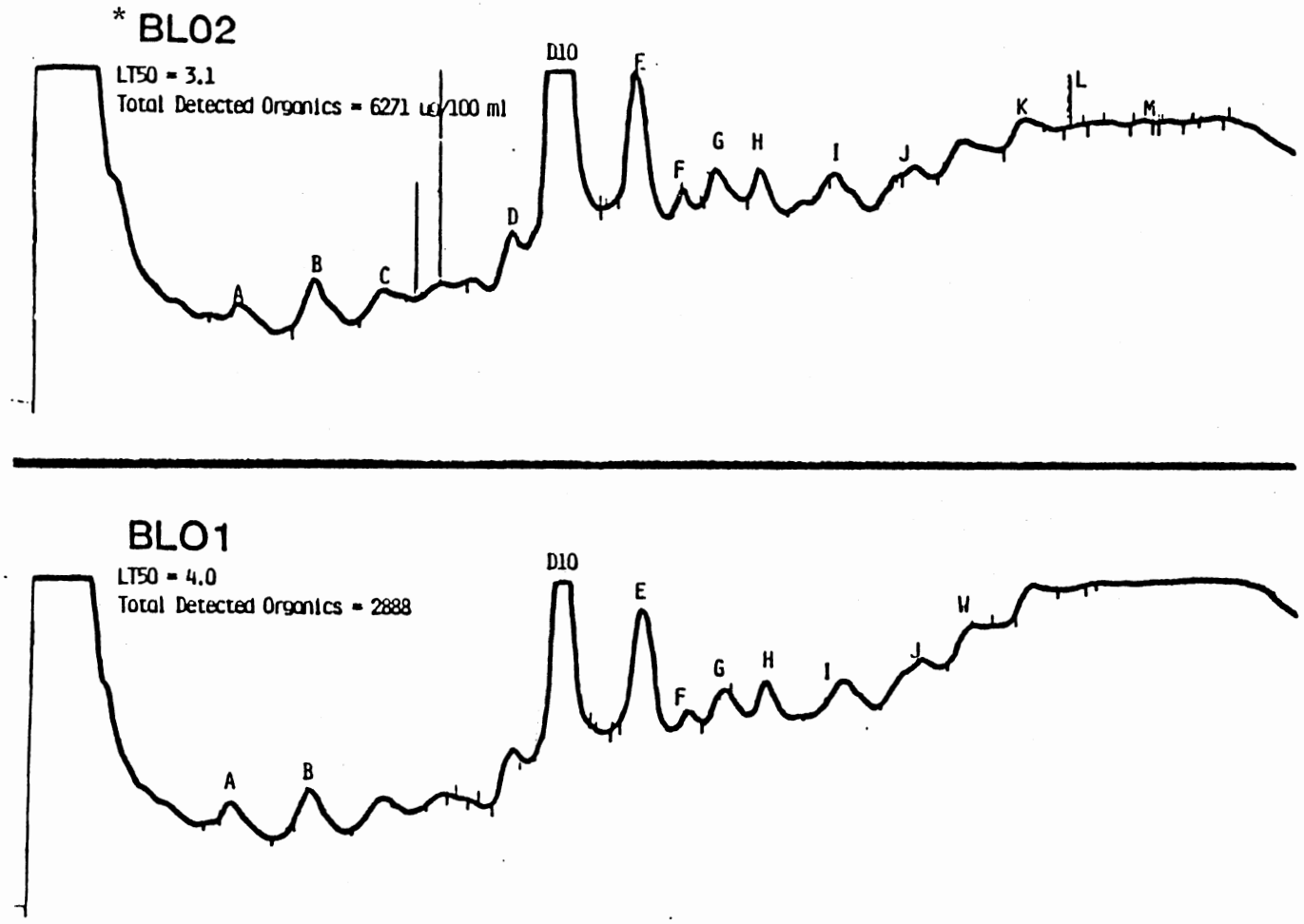


Figure 4. Chromatograms from October and August baseline elutriate extracts.

*Refer to page 42 for explanation of abbreviations.

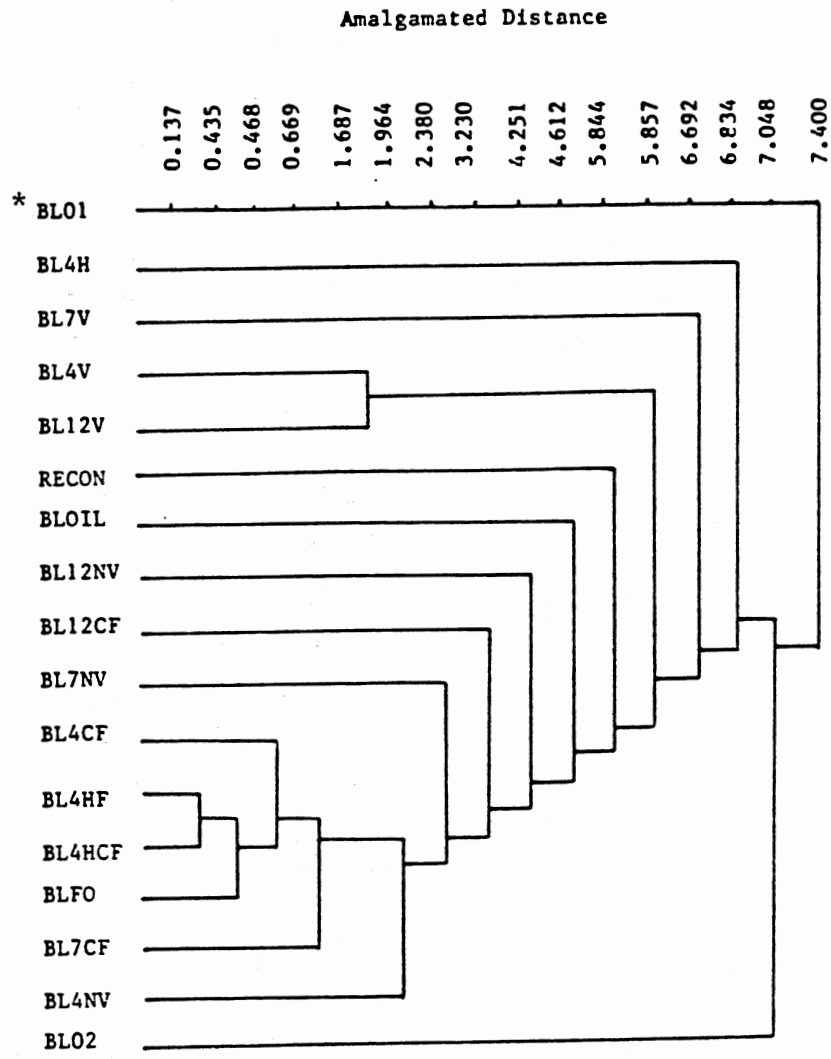


Figure 5. Dendogram for selected fraction similarities.
*Refer to page 42 for explanation of abbreviations.

BLO1	August baseline elutriate (unfractioned)
BLO2	October baseline elutriate (unfractioned)
BLFO	baseline filtered only
BL4CF	baseline treated with activated carbon and filtered at pH 4
BL7CF	baseline treated with activated carbon and filtered at pH 7
BL12CF	baseline treated with activated carbon and filtered at pH 12
BL4V	volatile from distillation at pH 4
BL7V	volatile from distillation at pH 7
BL12V	volatile from distillation at pH 12
BL4NV	non-volatile from distillation at pH 4
BL7NV	non-volatile from distillation at pH 7
BL12NV	non-volatile from distillation at pH 12
BL4H	clear layer removed from baseline heated at pH 4
BL4HF	clear layer removed from baseline heated at pH 4 then filtered
BLHFC	clear layer removed from baseline heated at pH 4 then treated with activated carbon and filtered
BLOIL	oily layer only after heating at pH 4
RECON	reconstituted water
SC	Stebler Creek control sediment elutriate

Figure 6. Explanation of Abbreviations for selected fractions.

CHAPTER V

SUMMARY

The objective of this study was to characterize the acutely lethal substances in sediment contaminated by oil refinery wastewater. The oil refinery wastewater was not normally toxic in standard Daphnia magna acute toxicity bioassays. However, the absence of benthic macroinvertebrates in the receiving stream indicated severe stress. Contaminated sediment samples were mixed with medium hard reconstituted water to obtain an elutriate which had LT50's ranging from three to four hours. Aliquots of sediment elutriate were subsequently treated by activated carbon adsorption, distillation, filtration, heat, and chelation. Static 48 hour Daphnia magna acute bioassays were used to evaluate the relative toxicities of the sediment elutriate fractions. Selected fractions were analyzed for nonpolar organic compounds using gas liquid chromatography. The acutely lethal substances in the sediment may be characterized as relatively non-volatile, weak organic acids. Toxicity was also partially attributed to physical causes such as oily substances interfering with respiration and movement.

LITERATURE CITED

Adams, W.J. 1987. In K.L. Dickson, A.W. Maki, and W. Brungs, eds., Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems. Perganon Press, Oxford. 450 pp. American Public Health Association, American Water Works Association/ Water Pollution Control Federation. 1980. Standard Methods for the Examination of Water and Wastewater, 15th ed.

Anderson-Carnahan, L. and D.I. Mount. 1987. Methods for toxicity reduction evaluations. EPA/600/1987. Duluth, Minnesota. 56 pp. Bahnick, D.A., W.A. Swenson, T.P. Markee, D.J. Call, C.A. Anderson and R.T. Morris. 1980. Development of bioassay procedures for defining pollution of harbor sediments. June 1981. Environmental Research Laboratory, Duluth, MN. EPA/600/S3-81-025.

Barth, D.S. and T.H. Starks. 1985. Sediment sampling quality assurance user's guide. August 1985. Environmental Monitoring Systems Laboratory, Las Vegas, NV. EPA/600/S4-85-048.

Buikema, A.L. Jr., J.G. Geiger, and D.R. Lee. 1980. Daphnia magna toxicity tests. In A.L. Buikema, Jr. and J. Cairns, Jr., eds. Aquatic Invertebrate Bioassays, Amer. Soc. Test. Mat., ASTM STP 715, 209pp.

Burks, S.L. 1982. Review of pollutants in petroleum refinery wastewaters and effect upon aquatic organisms. Environ. International. 7:271-283.

Chapman, P.M. 1986. Sediment quality criteria from the sediment quality triad: an example. Environ. Toxicol. Chem. 5:957-964.

Chapman, P.M., J.D. Propham, J. Griffen, D. Leslie, and J. Michaelson. 1987. Differentiation of physical from chemical tixicity in solid waste fish bioassays. Water, Air, and Soil Pollution. 33:295-308.

Chian, E.S.K., J.H. Reuter, and M. Giabbai. 1985. Isolation and concentration of organic substances from wateer using synthetic resins and graphitized carbon black. EPA-600/S1-84/025. Health Effects Research Laboratory, Research Triangle Park, NC.

Day, P.R. 1965. Particle fractionation and partical-size analysis. In C.A. Black, ed. Methods of Soil Analysis, Part 1, Physical and Mineralogical Properties. pp. 545-567.

Dean, H.H. and G.M. DeGraeve. 1986. A screen device to eliminate "floaters" in Daphnia magna toxicity tests. Environ. Toxicol. Chem. 5:1055-1057.

Dorris, T.C., S.L. Burks, M.R. Curd, G.R. Waller, and L.D. Broemeling. 1972. Toxic components in oil refinery effluents and their effect upon aquatic biota. Tech. Complet. Rep., OWRR B-017-Okla., U.S. Dept. Interior, Office of Water Resources Research. 113 pp.

Epler, J.L., B.R. Clark, C.-h. Ho, M.R. Guerin , and T.K. Rao. 1978. Short-term bioassay of complex organic mixtures: Part 11, Mutagenicity testing. In M.D. Waters, S. Nesnow, J.L. Huisingh, S. Sandher, and L. Claxton eds, Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. Plenum press, New York and London. 588 pp.

Gaudette, H.I., W.R. Flight, L. Toner, and D.W. Folger. 1974. An inexpenxsive titration method for the determination of organic carbon in recent sediments. J. Sedimentary Petrology. 44:249-253.

Guerin, M.R., B.R. Clark, C.H. Ho, J.L. Epler, and T.K. Rao. 1978. Short term bioassays of complex organic mixtures: Part 1, Chemistery. In M.D. Waters ed., Application of Short Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. Environ. Sci. Res. Vol. 15. 588 pp.

Jones, R.A. and G.F. Lee. 1978. Evaluation of the elutriate test as a method of predicting contaminant release during open water disposal of dredged sediment and environmental impact of open water dredged material disposal, Technical Report D-78-45. U.S. Army Corps of Engineers. Waterways Experiment Station, Vicksburg, MS. 217 pp.

Jones, R.A., G.M. Mariana, and G.F. Lee. 1981. Evaluation of the significance of sediment-associated contamination to water quality. In Utilizing Scientific Information in Environmental Quality Planning. Amer. Water Resources Assoc., Mineapolis, MN, pp. 34-45.

Karichoff, S.W., D.S. Brown, and T.A. Scott. 1979. Sorption of hydrophobic pollutants on natural sediments. Water Res. 13:241-248.

Lake, J.L., N. Rubinstein, and S. Pavignano. 1987. In K.L. Dickson, A.W. Maki, and W. Brungs, eds., Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems. Pergamon Press, Oxford. 450 pp.

Lee, F.G. and R.A. Jones. 1987. In K.L. Dickson, A.W. Maki, and W. Brungs, eds., Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems. Pergamon Press, Oxford. 450 pp.

Lowengart, G., A. Hall, G.F. Lee, C. Zarba, R. Zeller, and B. Murden. 1987. In K.L. Dickson, A.W. Maki, and W. Brungs, eds., Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems. Pergamon Press, Oxford. 450 pp.

Lopez-Avila, V., W.D. McKenzie, W.W. Sutton, R. Kaminsky. 1986. Application of chemical fractionation/aquatic bioassay procedure to hazardous waste site monitoring. EPA-600/S4-85/059. Environmental Monitoring Systems Laboratory, Las Vegas, NV.

Matthews, J.E. and L. Myers. 1976. Acute toxic effects petroleum refinery wastewaters on redear sunfish. Environmental Protection Agency. EPA 600/2-76-241. 62 pp.

Morgan, S.L., M.D. Walla, and J.C. Rogers. 1987. Measuring and displaying chromatographic similarity using cluster analysis. Chromatography. July:12-20.

Morrison, R.T. and R.N. Boyd. 1983. Organic Chemistry. 4th ed. Allyn and Bacon, Inc. Boston. 1307 pp.

Nebeker, A.V., M.A. Cairns, J.H. Gakstatter, K.W. Malueg, G.S. Schuytema and D.F. Krawczyk. 1984. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. Environ. Toxicol. Chem. 3:617-630.

Parkhurst, B.R., C.W. Gehrs, and I.B Rubin. 1979. Value of chemical fractionation for identifying the toxic components of complex aqueous effluents. Aquatic Toxicology, ASTM STP 667. L.L. Marking and R.A Kimerle, eds., American Society for Testing and Materials, pp. 122-130.

Peltier, W. and C.I. Weber. 1985. Methods for measuring the acute toxicity of effluents to aquatic organisms, 3rd ed., Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH. EPA/600/4-85/013.

Reece, C.H. 1983. Isolation and chemical characterization of petroleum refinery wastewater fractions acutely lethal to Daphnia magna. Ph.D. Dessertation. Oklahoma State University, Stillwater, OK. 86 pp.

Reece, C.H. and S.L. Burks. 1984. Isolation and chemical characterization of petroleum refinery wastewater fractions acutely lethal to Daphnia magna. Aquatic Toxicology and Hazard Assessment: Seventh Symposium, ASTM STP 854, R.D. Cardwell, R. Purdy, and R.C. Bahner, eds., American Society for Testing and Materials, Philadelphia, pp. 319-332.

Smith, G. 1987. Correlation between trace contaminant mixtures in complex effluents and structure of benthic macroinvertebrates. PhD Dissertation. Oklahoma State University, Stillwater, Oklahoma. pp. 219.

U.S Environmental Protection Agency/U.S. Army Corps of Engineers. 1977. Ecological evaluation of proposed discharge of dredged material into clean waters. Environmental Effects Laboratory, U.A. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS. 66 pp.

U.S. Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. Environmental Monitoring and Support Laboratory, Cincinnati, OH, EPA 600/4-79-020.

Veith, G.D., D.J. Call, and L.T. Brooke. 1983. Estimating the acute toxicity of narcotic industrial chemicals to fathead minnows. Aquatic Toxicology and Hazard Assessment: Sixth Symposium. ASTM STP 802, W.E. Bishop, R.D. Cardwell, and B.B. Heidolph, eds., American Society for Testing and Materials, Philadelphia. pp. 90 - 97.

Wolfe, D.A. 1987. In K.L. Dickson, A.W. Maki, and W. Brungs, eds., Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems. Perganon Press, Oxford. 450 pp.

Wolff, D.D. and M.L. Parsons. 1983. Pattern Recognition Approach to Data Interprtation, Plenum Press, New York. 105 pp.

APPENDIX A

CHROMATOGRAMS FROM SELECTED ELUTRIATE FRACTIONS

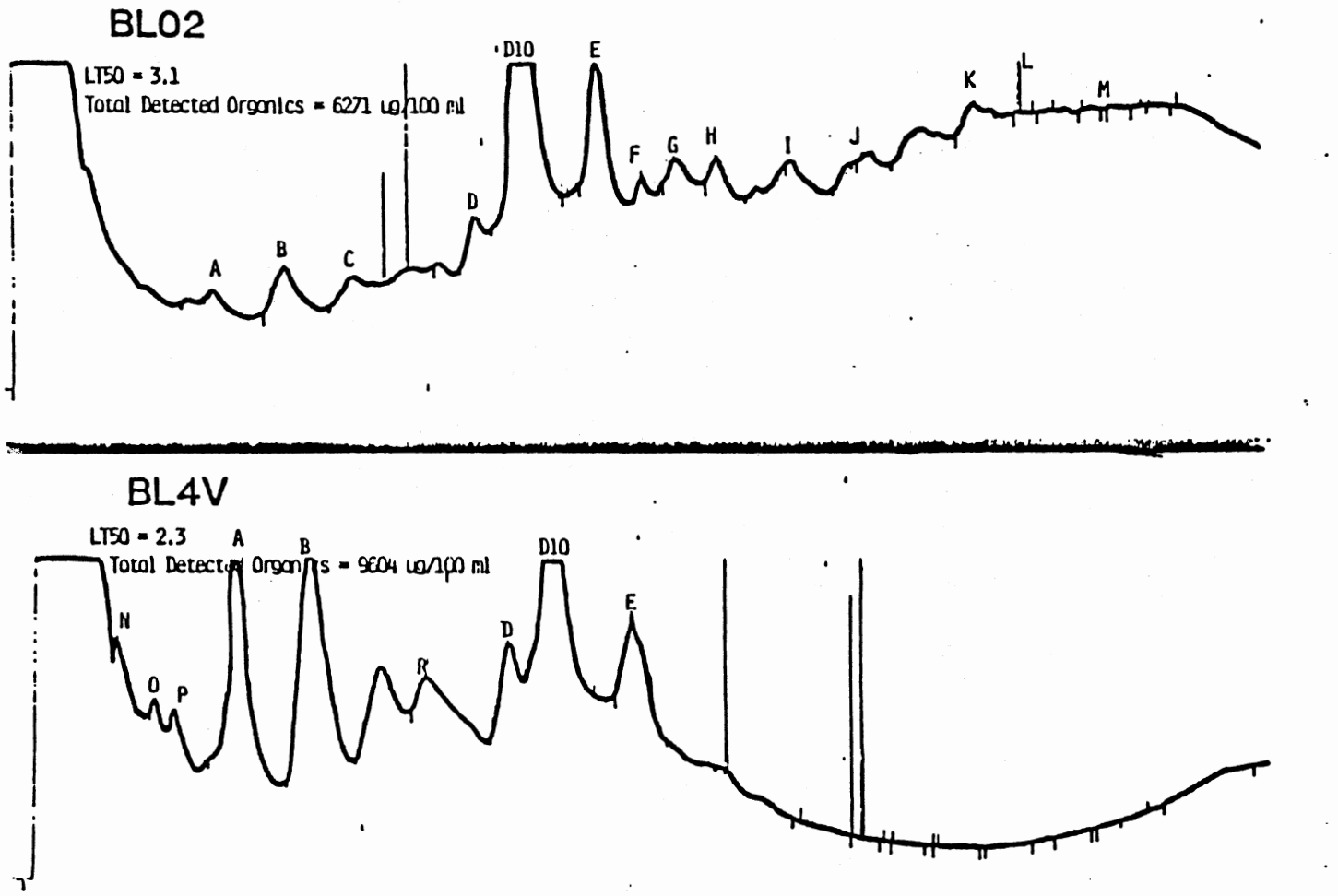


Figure 1A. October baseline and volatile fraction from pH 4 distillation.

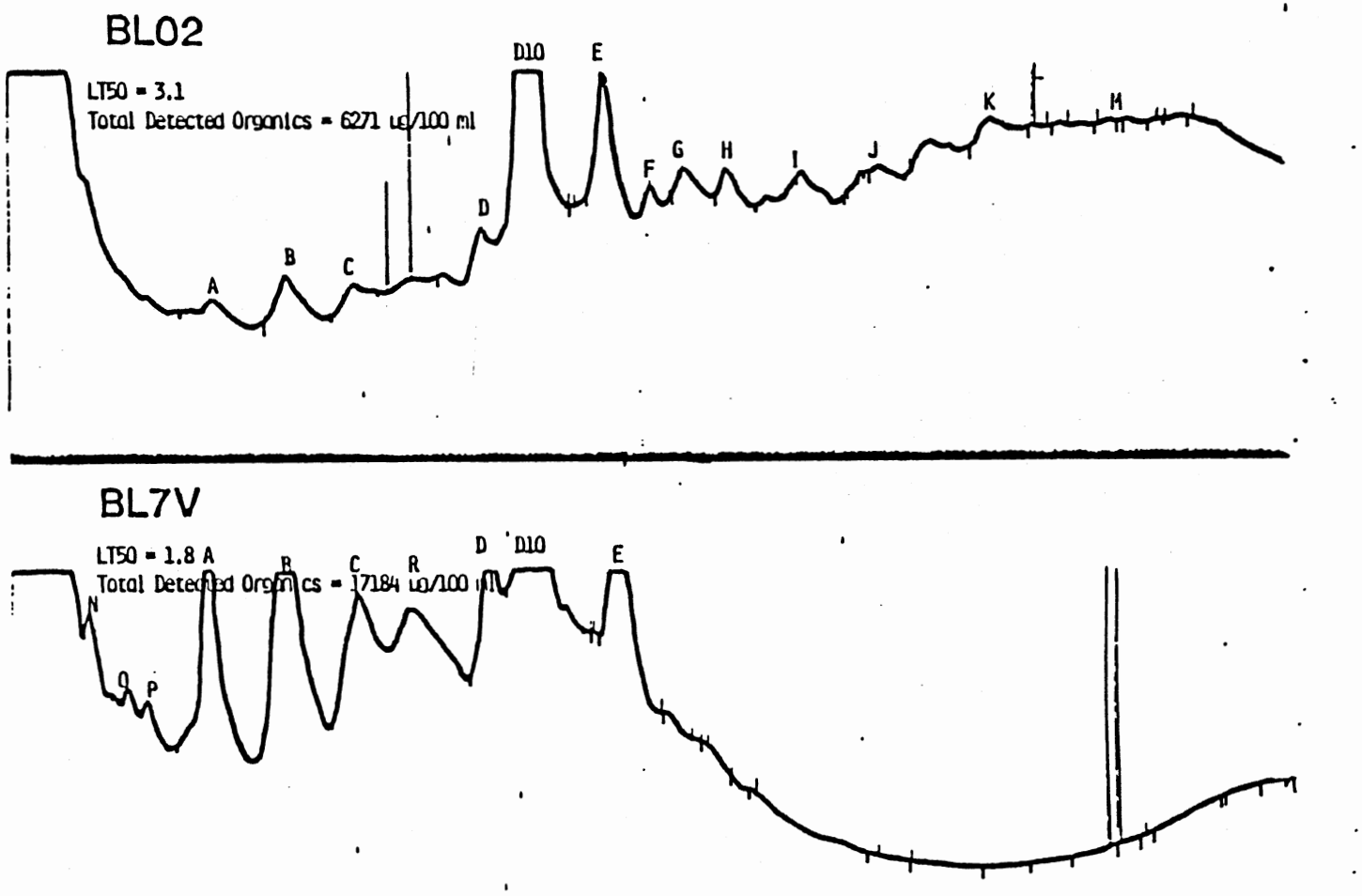


Figure 2A. October baseline and volatile fraction from pH 7 distillation.

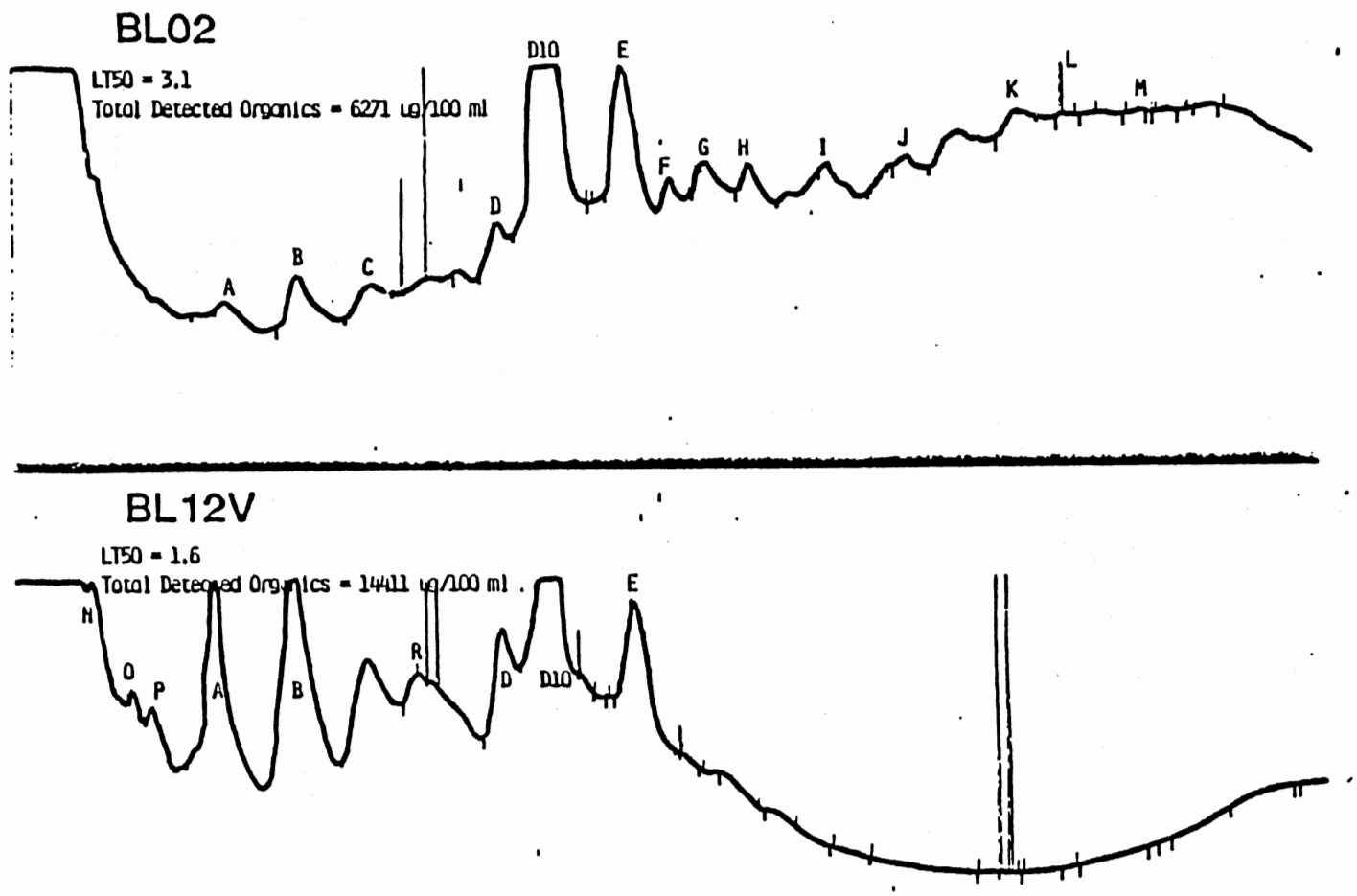


Figure 3A. October baseline and volatile fraction from pH 12 distillation.

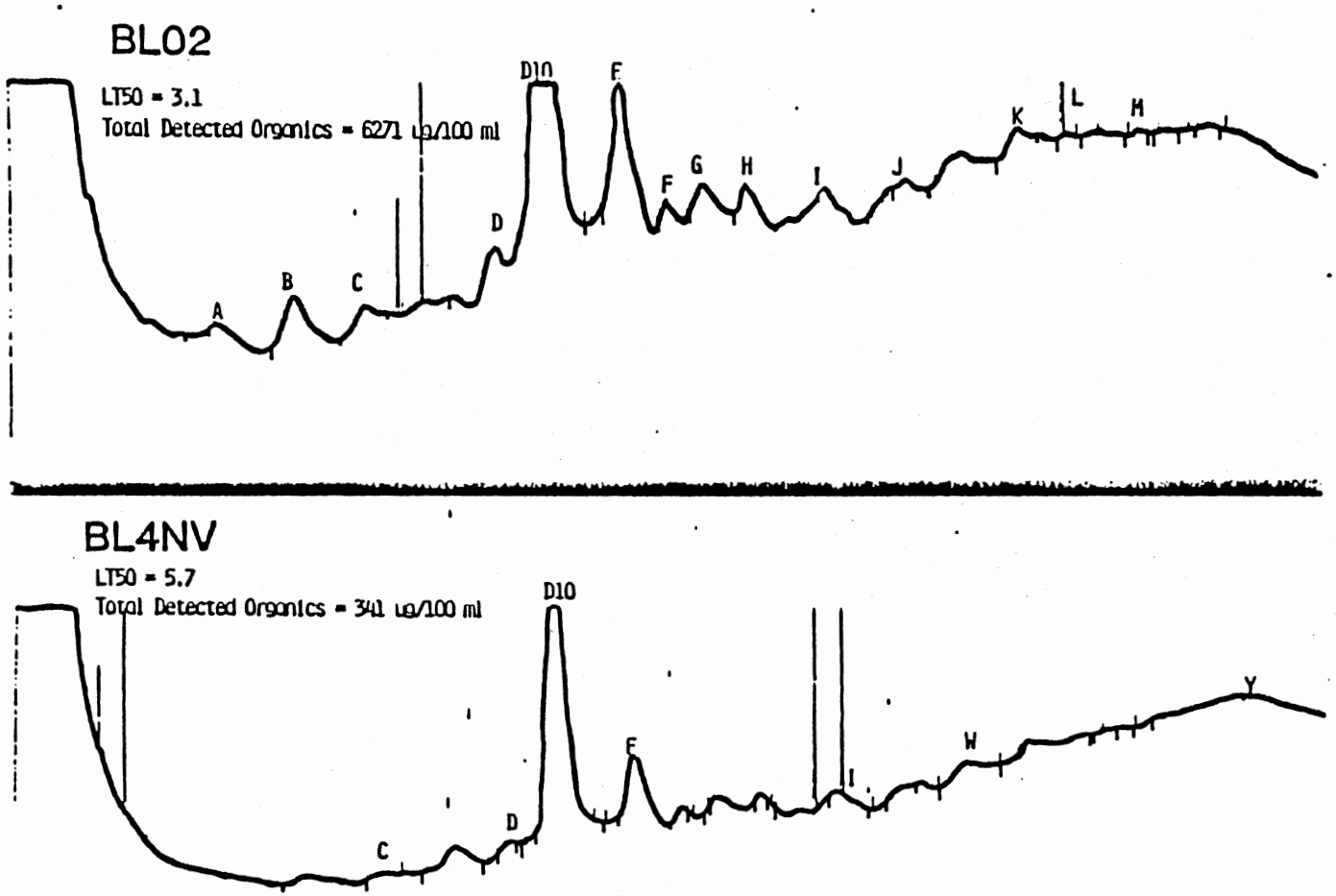


Figure 4A. October baseline and non-volatile fraction from pH 4 distillation.

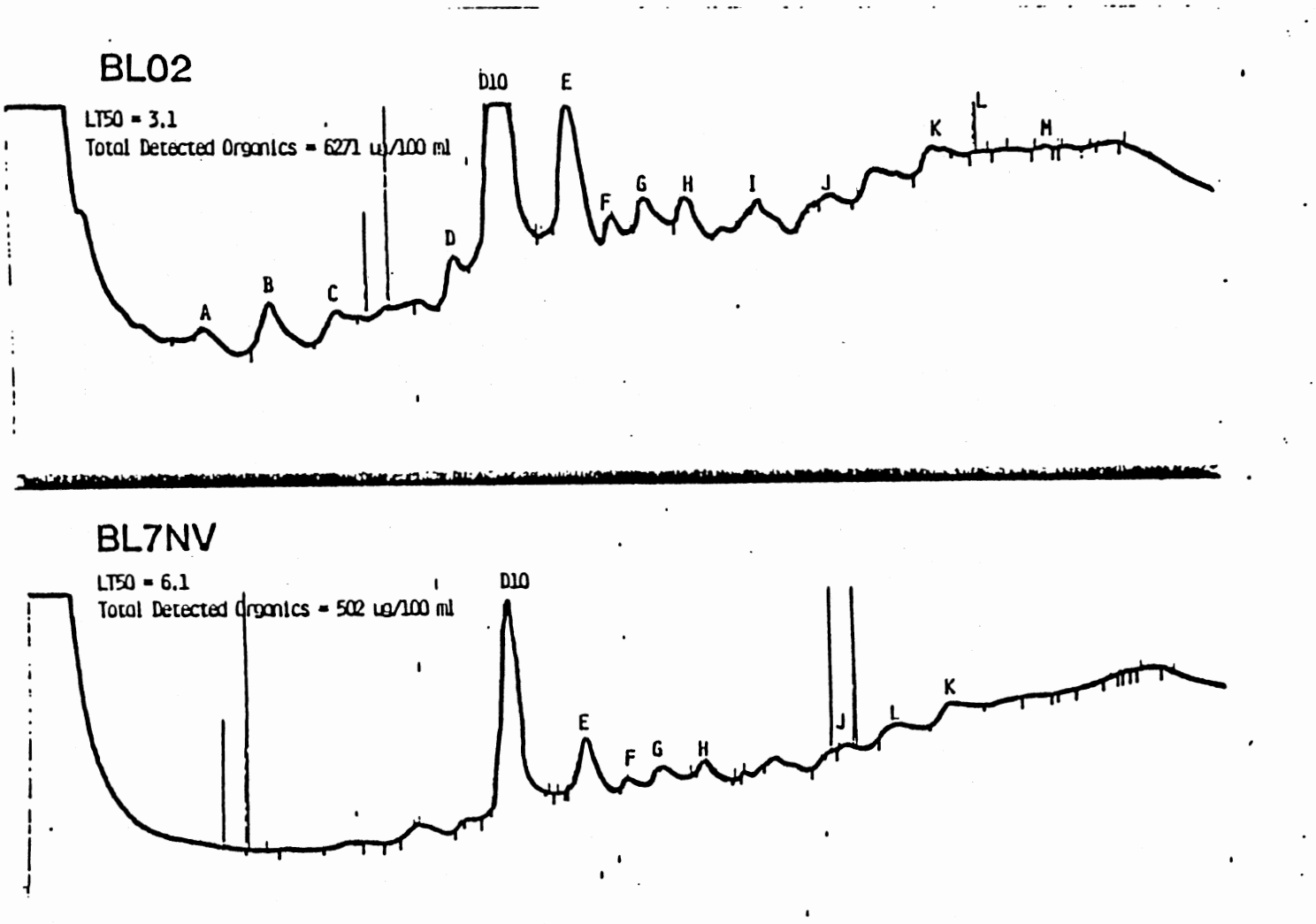


Figure 5A. October baseline and non-volatile fraction from pH 7 distillation.

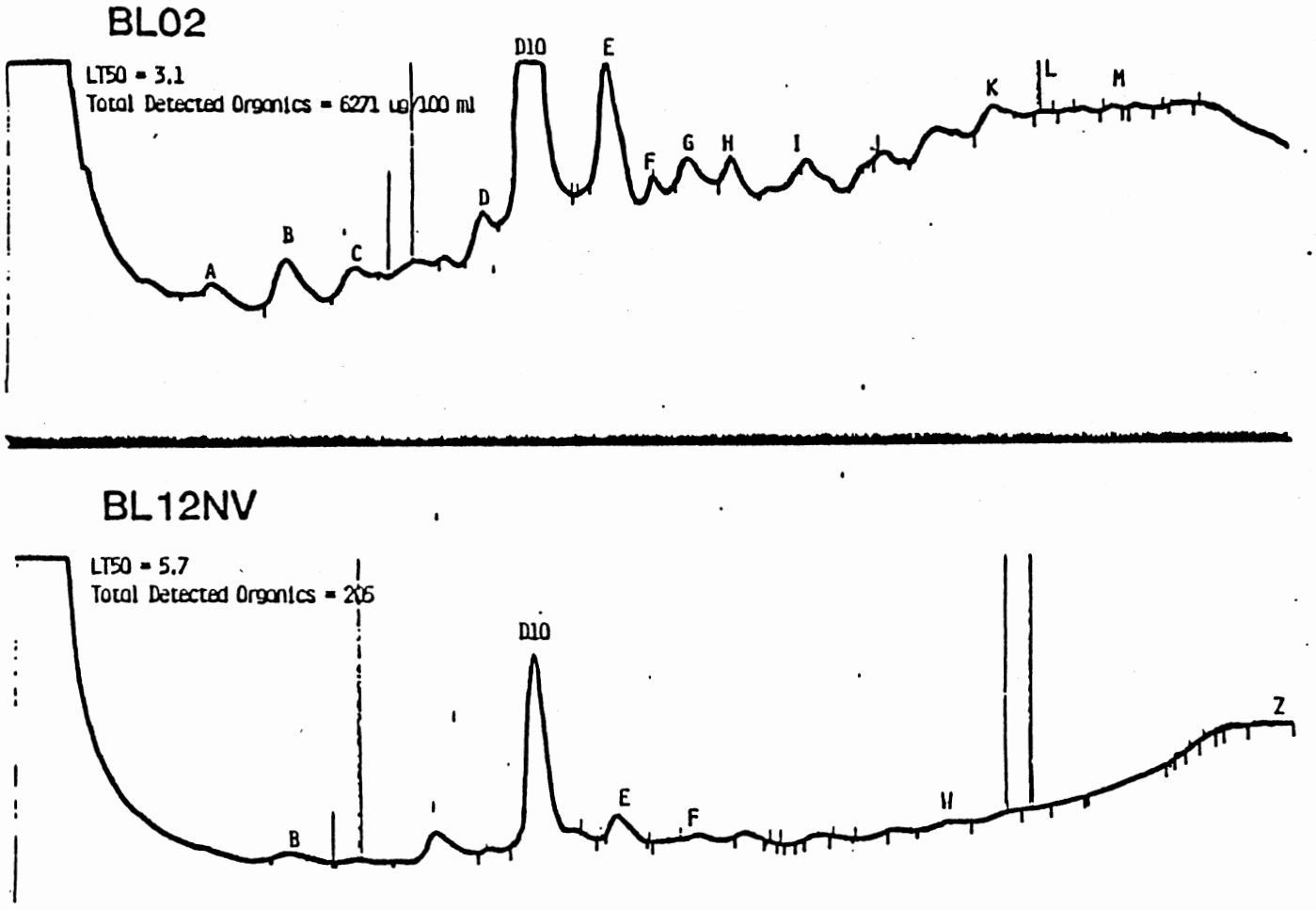


Figure 6A. October baseline and non-volatile fraction from pH 7 distillation.

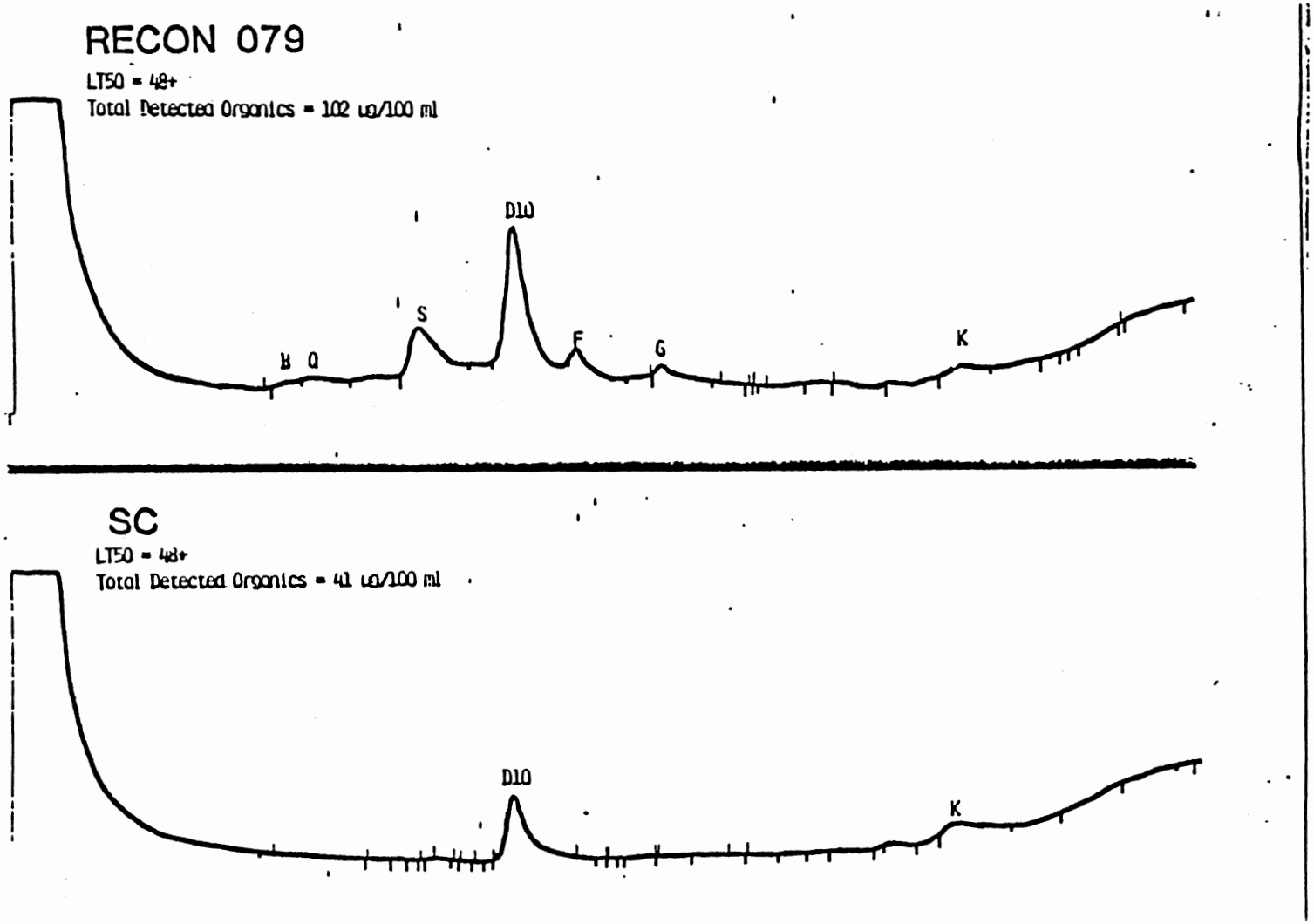


Figure 7A. Reconstituted water and Stebler Creek water (controls).

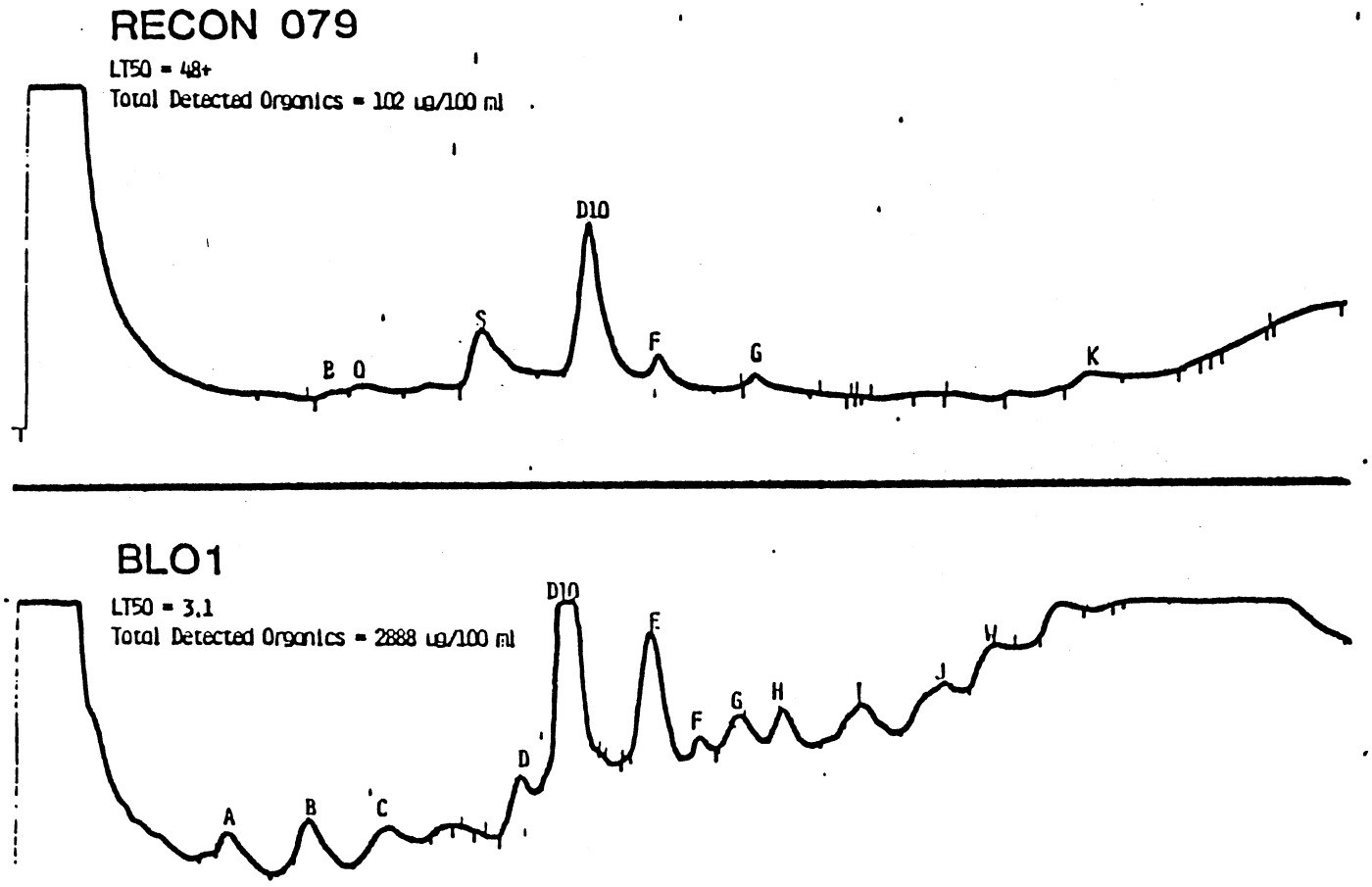


Figure 8A. Reconstituted water and August baseline.

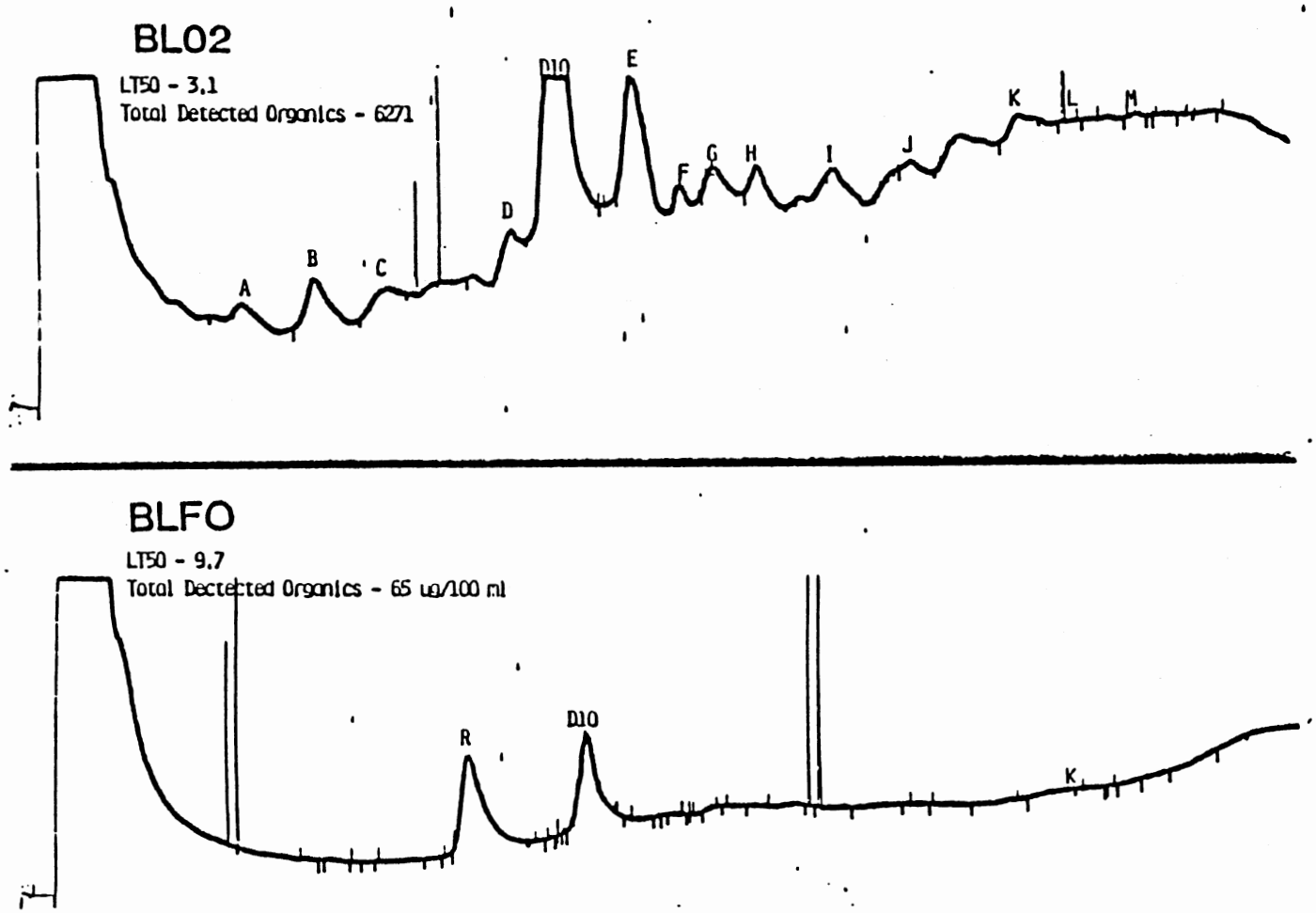


Figure 9A. October baseline and October filtered only fraction.

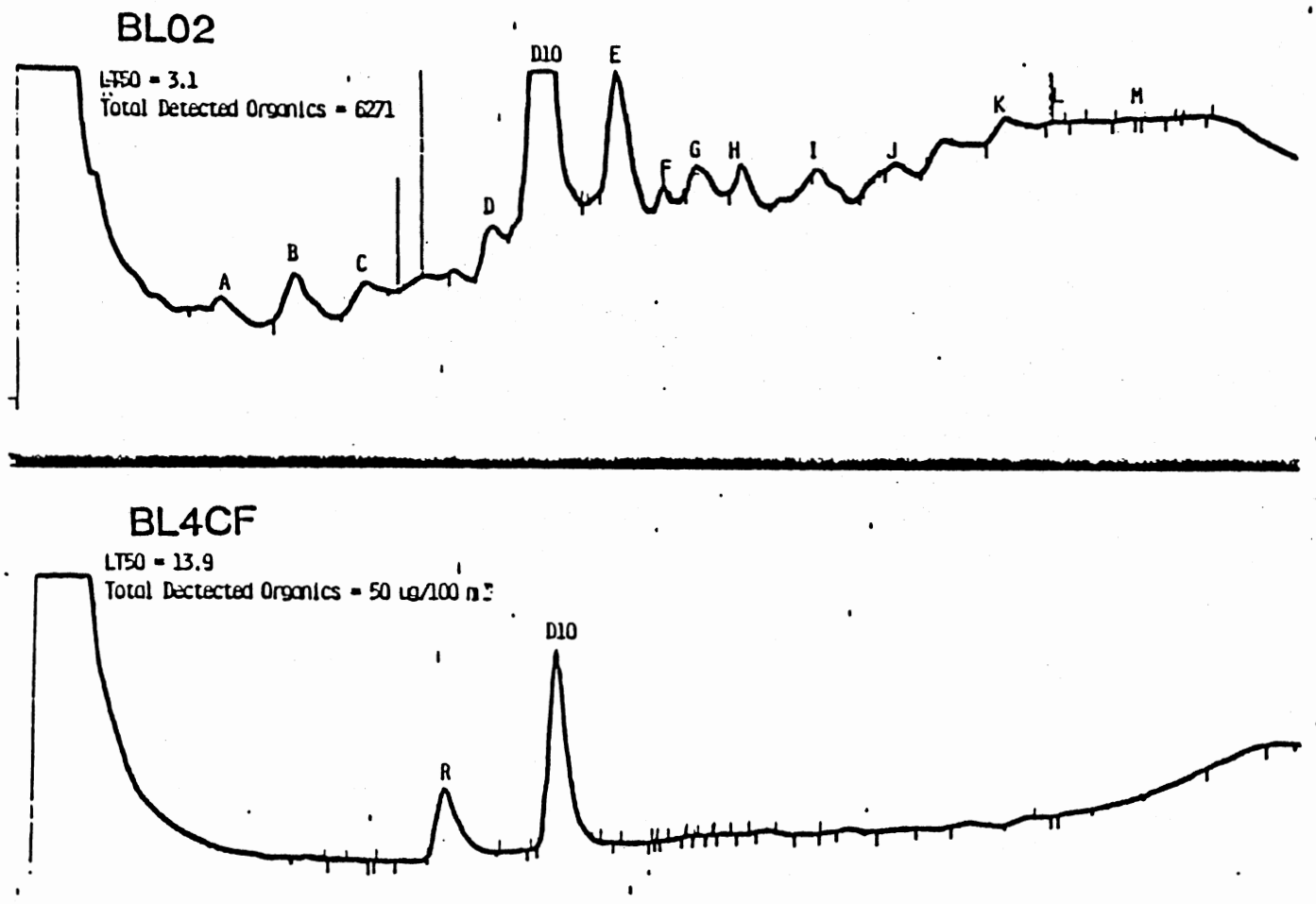


Figure 10A. October baseline and October activated carbon treated and filtered at pH 4 fraction.

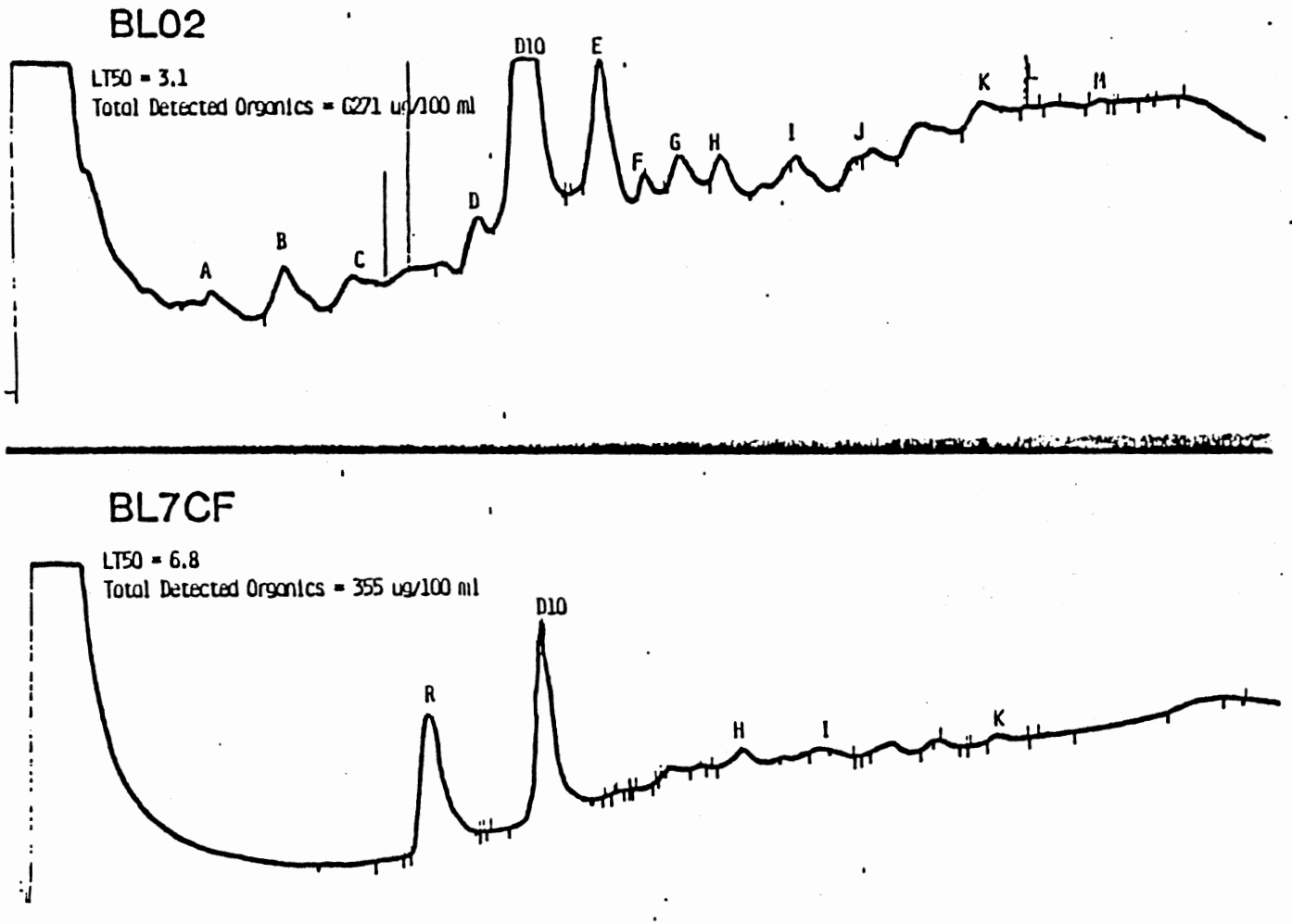


Figure 11A. October baseline and activated carbon treated and filtered at pH 7 fraction.

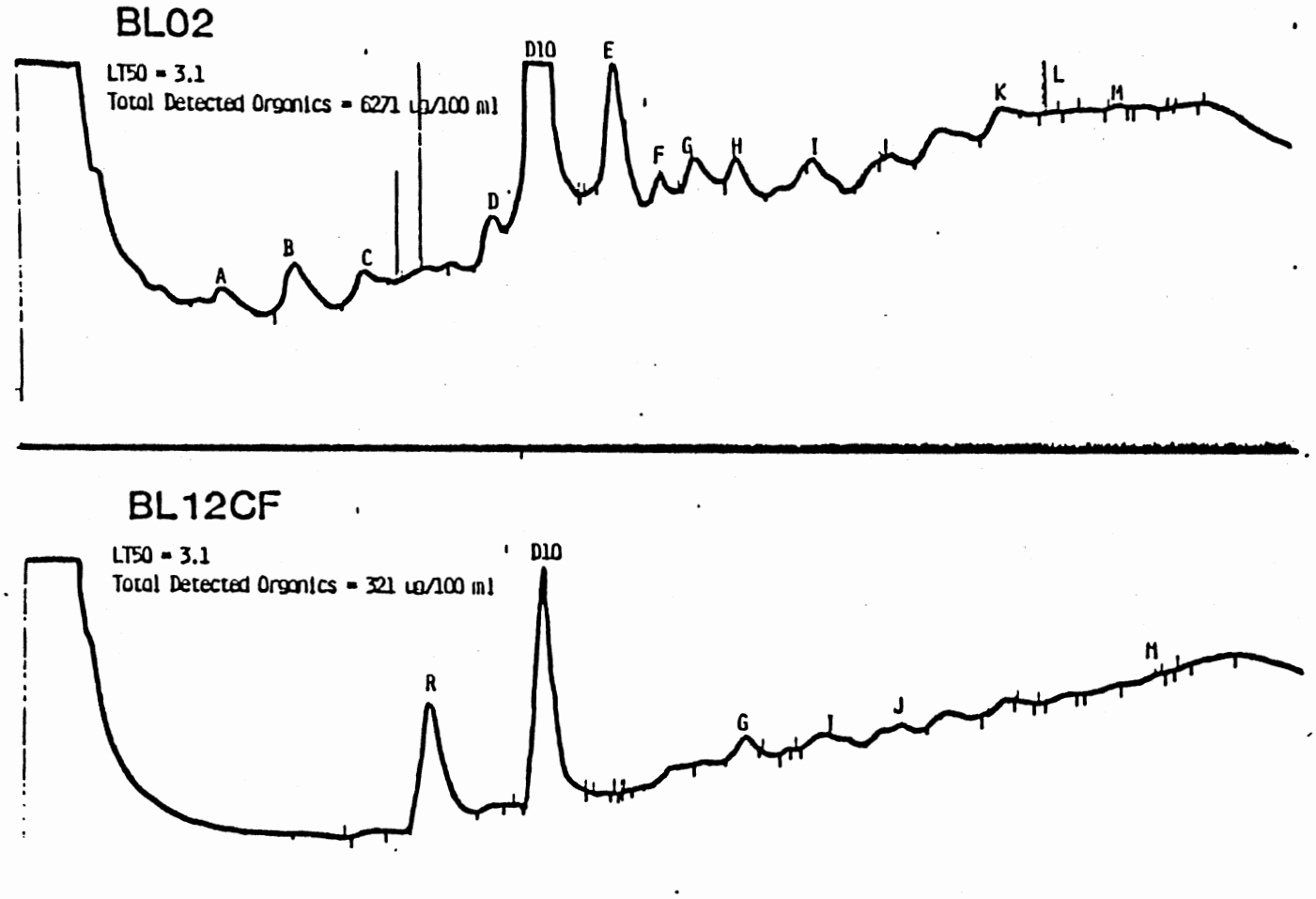


Figure 12A. October baseline and activated carbon treated and filtered at pH 12 fraction.

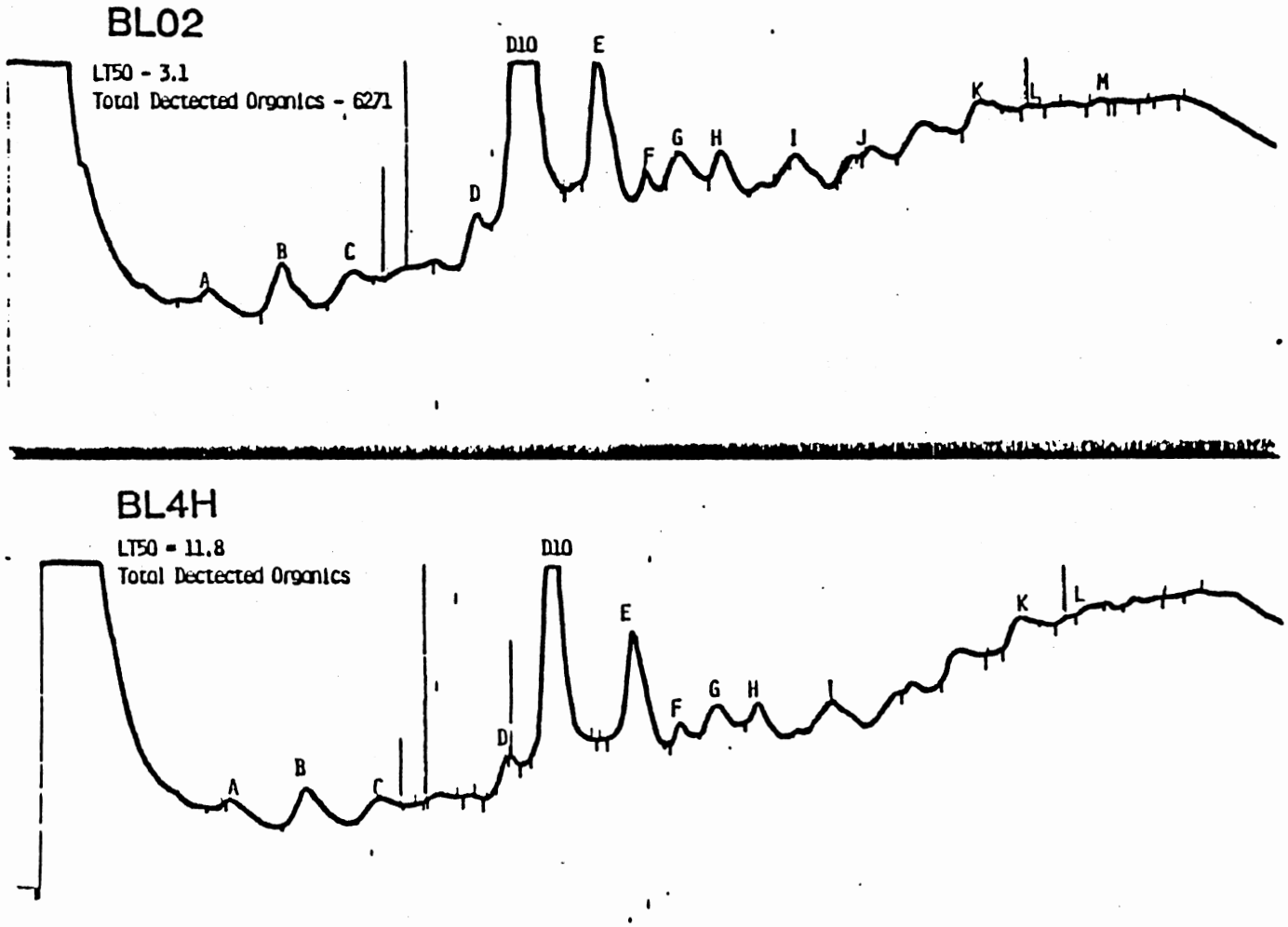


Figure 13A. October baseline and baseline heated at pH 4.

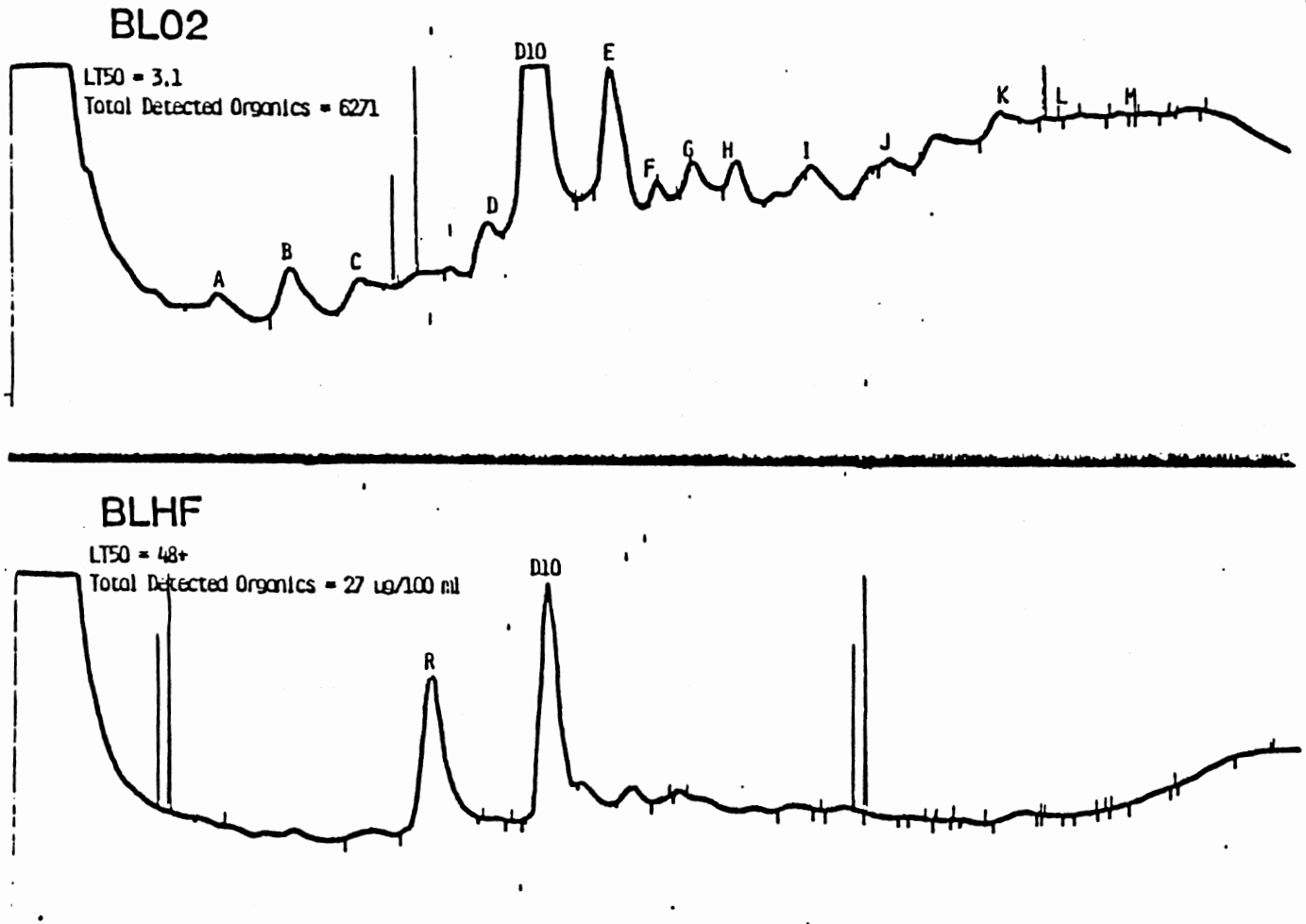


Figure 14A. October baseline and baseline heated and filtered at pH 4.

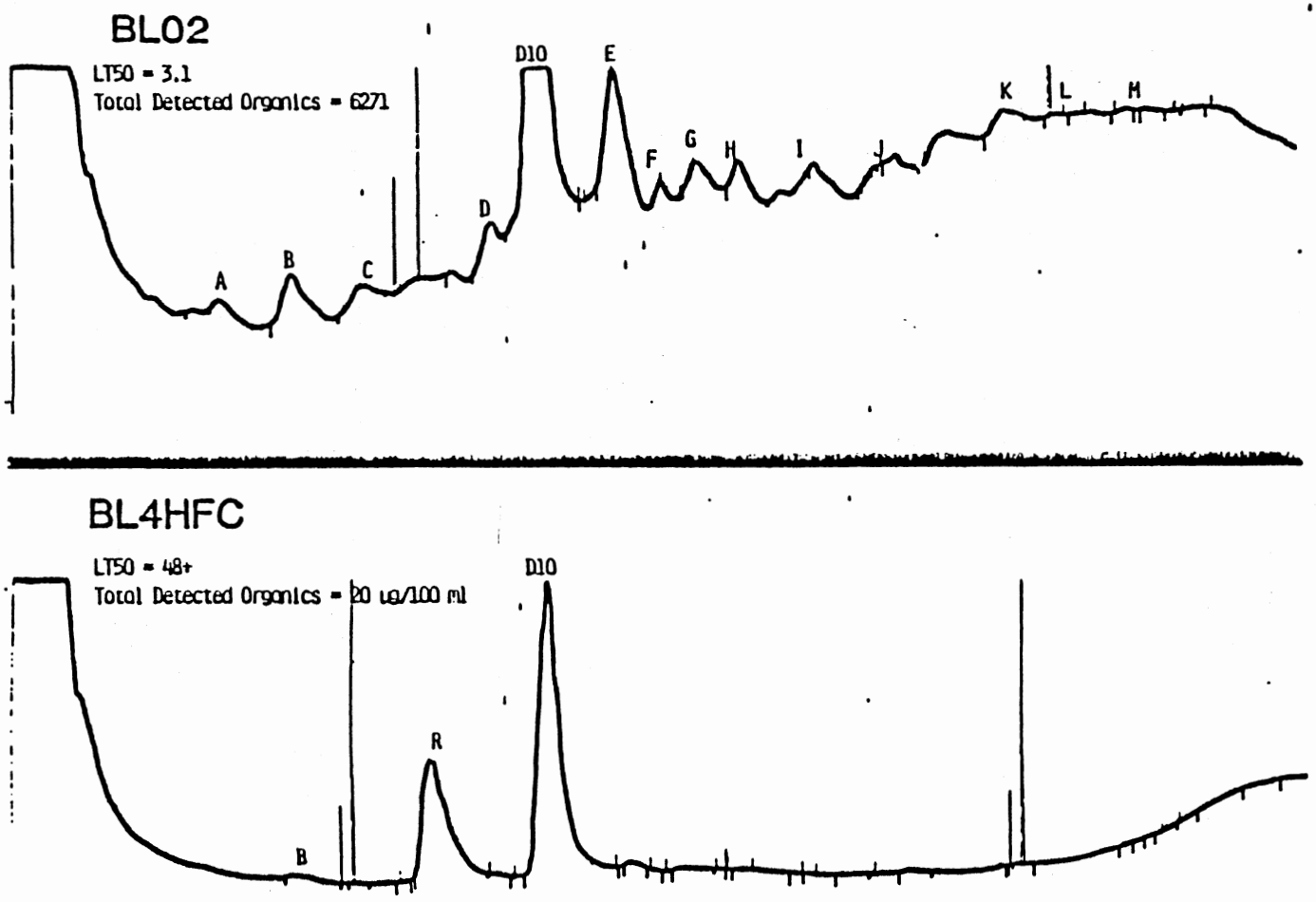


Figure 15A. October baseline and heated, filtered, and treated with activated carbon fraction.

APPENDIX B

QUANTITIES OF FRACTION COMPONENTS DETECTED BY GC

All quantities are in ug/100 ml.

RRT = relative retention time (D_{10})

peak	A	B	C	D	E	F
RRT	**0.420**	**0.563**	**0.689**	**0.911**	**1.125**	**1.208**
fraction						
BL02	242	315	157	230	678	206
BL4H	164	269	35	234	644	211
BL4nv	<8	<8	11	<8	84	<8
BL7nv	<8	<8	<8	<8	57	43
BL12nv	<9	31	<9	<9	50	40
BL4v	2039	2612	<75	800	1782	<101
BL7v	2427	3493	2250	1672	2457	<118
BL12v	2516	3138	<89	1302	2234	<118
BL4cf	<1.5	<1.5	<1.5	<1.5	<2.5	<2.5
BL7cf	<1.0	<1.0	<1.0	<1.0	<1.6	<1.6
BL12cf	<1.0	<1.0	<1.0	<1.0	<2.0	<2.0
BLfo	<1.6	<1.6	<1.6	<1.6	<2.2	<2.2
BL4hf	<1.2	<1.2	<1.2	<1.2	<1.6	<1.6
BL4hfc	<2.4	10	<2.4	<2.4	<3.2	<3.2
SC	<1.2	<1.2	<1.2	<1.2	<1.6	<1.6
RECON	<1.6	7	<1.6	<1.6	26	<2.2
SC4cf	<1.5	<1.5	<1.5	<1.5	<2.0	<2.0
SC7cf	<1.6	<1.6	<1.6	<1.6	<2.2	<2.2
SC12cf	<.9	<.9	<.9	<.9	<1.2	<1.2
SC4nv	<1.8	<1.8	<1.8	<1.8	<2.4	<2.4
SC7nv	<3.0	<3.0	<3.0	<3.0	<4.0	<4.0
SC12nv	<1.5	<1.5	<1.5	<1.5	<2.0	<2.0

Peaks A to F continued:

peak	A	B	C	D	E	F
RRT	**0.420**	**0.563**	**0.689**	**0.911**	**1.125**	**1.208**
fraction						
SC4v	<.7	<.7	<.7	<.7	<1.0	7
SC7v	<1.5	<1.5	<1.5	<1.5	<2.0	<2.0
SC12v	<2.1	<2.1	<2.1	<2.1	<2.8	<2.8
BL01	207	196	<35	184	541	<46
BL1oil	<43.8	23	<43.8	<43.8	<58	<58

Peaks G to L:

peak	G	H	I	J	K	L
RRT	1.25	1.342	1.478	1.618	1.809	1.888
fraction						
BL02	267	267	206	182	242	3049
BL4H	339	293	152	<47	234	538
BL4nv	<11	<11	122	<11	<11	<11
BL7nv	38	46	<11	213	51	54
BL12nv	<12	<12	<12	<12	<12	<12
BL4v	<101	<101	<101	<101	<101	<101
BL7v	<118	<118	<118	<118	<118	<118
BL12v	<118	<118	<118	<118	<118	<118
BL4cf	<2.0	7	<2.0	<2.0	<2.0	<2.0
BL7cf	<1.6	7	6	<1.6	6	<1.6
BL12cf	6	<2.0	8	7	<2.0	<2.0
BLfo	<2.2	<2.2	<2.2	<2.2	18	<2.2
BL4hf	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
BL4hfc	<3.2	<3.2	<3.2	<3.2	<3.2	<3.2
SC	26	<1.6	<1.6	<1.6	15	<1.6
RECON	19	<2.2	<2.2	<2.2	19	<2.2
SC4cf	7	<2.0	<2.0	<2.0	<2.0	<2.0
SC7cf	<2.2	<2.2	<2.2	<2.2	<2.2	<2.2
SC12cf	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
SC4nv	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
SC7nv	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0
SC12nv	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
SC4v	<1.0	<1.0	<1.0	7	<1.0	<1.0
SC7v	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

Peaks G to L continued:

peak	**G**	**H**	**I**	**J**	**K**	**L**
RRT	**1.25**	**1.342**	**1.478**	**1.618**	**1.809**	**1.888**
fraction						
SC12v	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8
BL01	380	299	391	368	<46	<46
BL1oil	44	47	51	<58	45	<58

Peaks M to R:

peak	M	N	O	P	Q	R
RRT	2.037	0.213	0.280	0.312	0.605	0.747
fraction						
BL02	230	<36	<36	<36	<36	<36
BL4H	<47	<35	<35	<35	<35	339
BL4nv	<11	<8	<8	<8	<8	<8
BL7nv	<11	<8	<8	<8	<8	<8
BL12nv	<12	<9	<9	<9	<9	<9
BL4v	<101	529	483	483	75	876
BL7v	<118	622	533	622	<89	2250
BL12v	<118	622	533	622	<89	444
BL4cf	19	<1.5	<1.5	<1.5	<1.5	24
BL7cf	<1.6	<1.0	<1.0	<1.0	<1.0	336
BL12cf	6	<1.0	<1.0	<1.0	<1.0	27
BLfo	19	<1.6	<1.6	<1.6	<1.6	28
BL4hf	<1.6	<1.2	<1.2	<1.2	<1.2	27
BL4hfc	<3.2	<2.4	<2.4	<2.4	<2.4	10
SC	<1.6	<1.2	<1.2	<1.2	<1.2	<1.2
RECON	<2.2	<1.6	<1.6	<1.6	8	<1.6
SC4cf	<2.0	<1.5	<1.5	<1.5	<1.5	<1.5
SC7cf	<2.2	<1.6	<1.6	<1.6	<1.6	<1.6
SC12cf	<1.2	<0.9	<0.9	<0.9	<0.9	4
SC4nv	<2.4	<1.8	<1.8	<1.8	<1.8	28
SC7nv	<4.0	<3.0	<3.0	<3.0	<3.0	<3.0
SC12nv	<2.0	<1.5	<1.5	<1.5	<1.5	42
SC4v	<1.0	<0.7	<0.7	<0.7	<0.7	<0.7
SC7v	<2.0	<1.5	<1.5	<1.5	<1.5	4

Peaks M to R continued:

peak	M	N	O	P	Q	R
RRT	**2.037**	**0.213**	**0.280**	**0.312**	**0.605**	**0.747**
fraction						
SC12v	<2.8	22	<2.1	<2.1	<2.1	<2.1
BL01	<46	<35	<35	<35	<35	<35
BL1oil	<58	<43.8	<43.8	<43.8	<43.8	<43.8

Peaks S to X:

peak	S	T	U	V	W	X
RRT	0.811	0.951	1.132	1.570	1.674	1.931
fraction						
BL02	<36	<36	<60	<48	<48	<48
BL4H	<35	<35	<58	<47	<47	164
BL4nv	<8.0	<8.0	<11.0	<11.0	43	<11.0
BL7nv	<8.0	<8.0	<11.0	<11.0	<11.0	<11.0
BL12nv	<9.0	<9.0	<15.0	<12.0	<12.0	<12.0
BL4v	<75	<75	<126	<101	<101	<101
BL7v	<89	858	<148	<118	<118	<118
BL12v	<89	<89	<148	<118	<118	<118
BL4cf	<1.5	<1.5	<2.5	<2.0	<2.0	<2.0
BL7cf	<1.0	<1.0	<2.0	<1.6	<1.6	<1.6
BL12cf	<1.0	<1.0	<2.0	<2.0	<2.0	<2.0
BLfo	<1.6	<1.6	<2.7	<2.2	<2.2	<2.2
BL4hf	<1.2	<1.2	<2.0	<1.6	<1.6	<1.6
BL4hfc	<2.4	<2.4	<4.0	<3.2	<3.2	<3.2
SC	<1.2	<1.2	<2.0	<1.6	<1.6	<1.6
RECON	23	<1.6	<2.7	<2.2	<2.2	<2.2
SC4cf	<1.5	<1.5	<2.5	<2.0	<2.0	<2.0
SC7cf	<1.6	<1.6	<2.7	<2.2	<2.2	<2.2
SC12cf	4	<.9	<1.5	<1.2	<1.2	<1.2
SC4nv	28	<1.8	<3.0	<2.4	<2.4	<2.4
SC7nv	<3.0	<3.0	<5.0	<4.0	<4.0	<4.0
SC12nv	42	<1.5	<2.5	<2.0	<2.0	<2.0
SC4v	<.7	<.7	<1.2	<1.0	6	<1.0

Peaks S to X continued:

peak	S	T	U	V	W	X
RRT	**0.811**	**0.951**	**1.132**	**1.570**	**1.674**	**1.931**
fraction						
SC7v	4	<1.5	<2.5	<2.0	<2.0	<2.0
SC12v	19	<2.1	<3.5	<2.8	<2.8	<2.8
BL01	<35	<35	<57	<46	322	<46
BL1oil	<43.8	<43.8	<73	41	48	<58

Peaks Y, Z, Total Detected Organics, and LT50's:

peak	**Y**	**Z**	**Tot. Det.**	
RRT	**2.181**	**2.363**	**Organics**	**LT50**
fraction				
BL02	<48	<48	6271	3.1
BL4H	<47	<47	3616	11.8
BL4nv	81	<11	341	5.7
BL7nv	<11	<11	502	6.1
BL12nv	<12	40	205	5.7
BL4v	<101	<101	9604	2.3
BL7v	<118	<118	17184	1.8
BL12v	<118	<118	11411	1.6
BL4cf	<2	<2	50	13.9
BL7cf	<1.6	<1.6	355	6.8
BL12cf	267	<2	321	5.7
BLfo	2.2	2.2	65	9.7
BL4hf	1.6	1.6	27	48+
BL4hfc	<3.2	<3.2	20	48+
SC	<1.6	<1.6	41	48+
RECON	<2.2	<2.2	102	48+
SC4cf	<2	<2	7	48+
SC7cf	<2.2	<2.2	<2.7	48+
SC12cf	<1.2	<1.2	4	48+
SC4nv	<2.4	<2.4	28	48+
SC7nv	<4	<4	<5	48+
SC12nv	<2	<2	42	48+
SC4v	<1	<1	20	48+

Peaks Y, Z, Total Detected Organics, and LT50's continued:

peak RRT	Y 2.181	Z 2.363	Tot. Det. Organics	LT50
fraction				
SC7v	<2	<2	4	48+
SC12v	<2.8	<2.8	41	48+
BL01	<46	<46	<46	4.0
BL1oil	<58	<58	376	1.6

APPENDIX C

RESULTS FROM AUGUST CHELATION

0.01 M EDTA added to 5 ml elutriate	LT50 (hours)
0.05	6.3
0.15	7.2
0.25	4.9
0.35	6.4
0.45	2.8
0.55	2.8
0.65	2.8
0.75	2.8
0.85	2.8
0.95	2.8

0.1 M EDTA added to 5 ml elutriate	LT50 (hours)
0.05	6.4
0.15	2.5
0.25	2.5
0.35	1.1
0.45	1.2
0.55	1.2
0.65	1.3
0.75	1.3
0.85	1.1
0.95	1.3

.01 M EDTA added to 5 ml reconstituted water	LT50 (hours)
0.05	9.2
0.15	9.2
0.25	9.2
0.35	9.2
0.45	7.3
0.55	6.4
0.65	11.3
0.75	6.8
0.85	13.8
0.95	7.3

APPENDIX D

LT50 TABLES WITH 95% CONFIDENCE INTERVALS FOR AUGUST AND OCTOBER FRACTIONS, EXCEPT CHELATION AND INCLUDING DEGRADATION

Test Component	Base-line	Fil-tered	fraction: Activated Carbon and filtration			fraction: Distillation volatile			fraction: Distillation non-volatile		
			pH 4	pH 7	pH 12	pH 4	pH 7	pH 12	pH 4	pH 7	pH 12
Baseline elutriate .	4	29.77	30.62	13.86	12.30	1.18	1.29	1.28	3.32	3.05	3.05
*95% Conf. interval	2-8	24-28	24-28	8-24	8-24	1-1.5	1-1.5	5-2	2-4	2-4	2-4
Control elutriate	48+	48+	48+	48+	48+	48+ 5%	48+	48+	48+	48+	48+
*95% Conf. interval	NM	NM	NM	NM	NM		10%	NM	NM	NM	NM
Centrifuged Reconstituted Water	48+	48+	48+	48+	48+	48+	48+	48+	1.87	48+	48+
*95% Conf. interval	NM	NM	NM	NM	NM	20%	10%	45%	1.5-4	NM	NM
Reconstituted Water	48+	48+	48+	48+	48+		48+			48+	
*95% Conf. interval	NM	NM	NM	NM	NM		10%			5%	

* If the LT50 is greater than 48 hours the 95% confidence interval cannot be determined and the percent mortality at 48 hours is listed.

NM = no mortality

LT50 Table for August

Test Component	fraction: heat at pH 4	heat carb. filt.	no oil layer	no oil layer filt.	no oil layer filt. carb.	oily layer only	oily layer plus recon.
Baseline elutriate	2.8	31.5	33.9	31.5	48+	1.6	9.7
*95% Conf. interval	2-4	24-48	24-48	24-48	NM	1-2	4-24
Control elutriate	48+						
95% Conf. interval	NM						
Reconstituted Water	48+	48+	48+	48+	48+	48+	48+
95% Conf. interval	NM	NM	NM	NM	NM	NM	NM

Test Component	fraction: Day 2		Day 4		Day 8		Day 24	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Baseline elutriate	3.0	3.7	2.7	2.5	3.4	3.1	16.3	20.9
95% Conf. interval	2-4	2-8	2-4	2-4	2-48	2-4	8-24	8-48
Control elutriate	26.7	48+	13.9	48+	7.5	48+	18.7	48+
95% Conf. interval	8-48	NM	4-inf	NM	4-24	NM	8-48	NM
Centrifuged Reconstituted Water	48+	48+	48+	48+	48+	48+	48+	48+
95% Conf. interval	20%	5%	NM	10%	35%	40%	NM	NM
Reconstituted Water	48+	48+	48+	48+	48+	48+	48+	48+
95% Conf. interval	5%	NM	50%	5%	5%	5%	NM	NM

*If LT50 = 48+ the 95% Conf. interval cannot be determined and the percent mortality at 48 hours is listed. NM = no mortality

LT50 Table for August

Test Component	Base-line	Base-line Filt.	fraction: Activated Carbon and Filtration			fraction: Distillation Volatile			fraction: Distillation Non-Volatile		
			pH 4	pH 7	pH 12	pH 4	pH 7	pH 12	pH 4	pH 7	pH 12
Baseline elutriate	3.1	9.7	13.9	6.8	5.7	2.3	1.8	1.6	5.7	6.1	5.7
95% Conf. interval	2-4	4-24	8-24	4-24	4-8	1.5-8	1.5-4	1-2	4-8	4-8	4-8
Control elutriate	48+	48+	48+	48+	48+	48+	48+	48+	48+	48+	48+
95% Conf. interval	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
Reconstituted Water	48+	48+	48+	48+	48+	48+	48+	48+	48+	48+	48+
95% Conf. interval	NM	NM	10%	NM	NM	NM	NM	NM	NM	NM	NM

*If LT50 = 48+ the 95% Conf. interval cannot be determined and the percent mortality at 48 hours is listed. NM = no mortality

LT50 Table for October

	fraction		
Test Component	heated pH 4	heated pH 4 and Filtered	heated pH 4 and Filt. and Carbon
Baseline elutriate	11.8	48+	48+
95% Conf. interval	8-24	5%	5%
Control elutriate			
95% Conf. interval			
Reconstituted Water	48+	48+	48+
95% Conf. interval	NM	NM	NM

*If LT50 = 48+ the 95% Conf. interval cannot be determined and the percent mortality at 48 hours is listed. NM = no mortality

LT50 Table for October

VITA

Sherry Ham Ratzlaff

Candidate for the Degree of

Master of Science

Thesis: CHARACTERIZATION OF SEDIMENT CONTAMINANTS ACUTELY LETHAL TO DAPHNIA MAGNA

Major Field: Zoology

Biographical:

Personal Data: Born in Roanoke, Virginia, October 11, 1955, the daughter of Evelyn B. and Kenneth H. Turner. Married to John Allen Ratzlaff on December 29,1987.

Education: Graduated from William Fleming High School, Roanoke, Virginia, in June 1975; received Associate of Science Degree in Science from Virginia Western Community College in May, 1982; received Bachelor of Science Degree in Biology from Radford University, Radford, Virginia in May 1984; completed requirements for the Master of Science degree at Oklahoma State University in July, 1988.

Professional Experience: Research Assistant, Virginia Fish and Game Commission, May 1984 to January 1985; Graduate Research Assistant, Oklahoma Fish and Wildlife Research Unit, Oklahoma State University, August 1985 to October 1986; Graduate Teaching Assistant, Oklahoma State Universisty,January 1987 to December 1987; Graduate Research Assistant, Water Quality Research Laboratory, Oklahoma State University, January 1988 to March 1988.